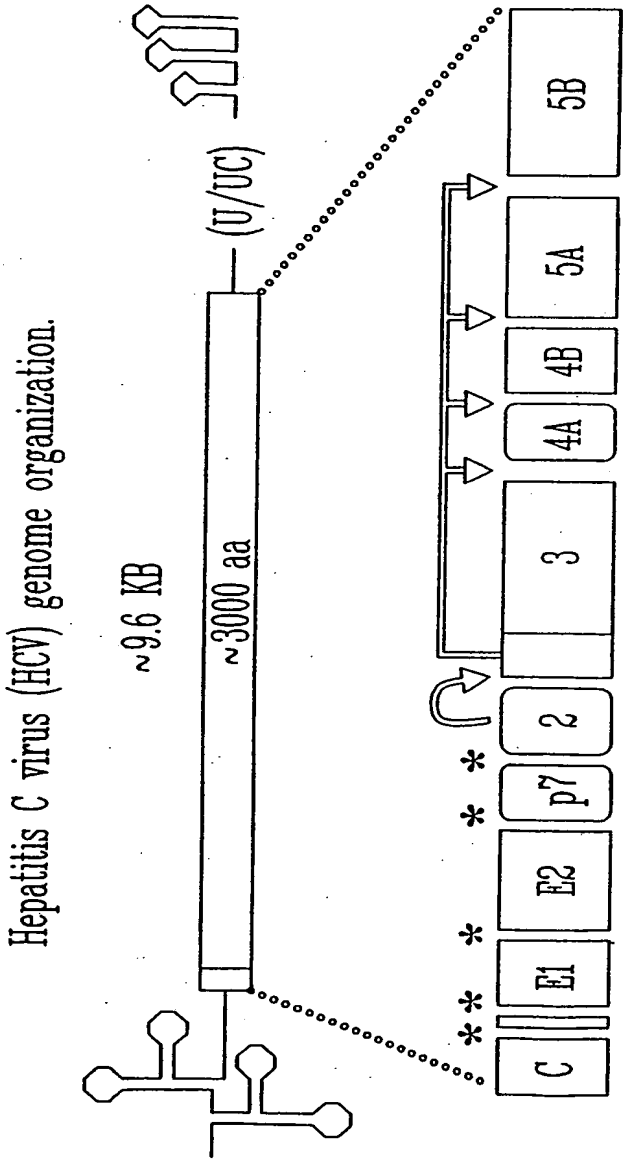


Fig. 1

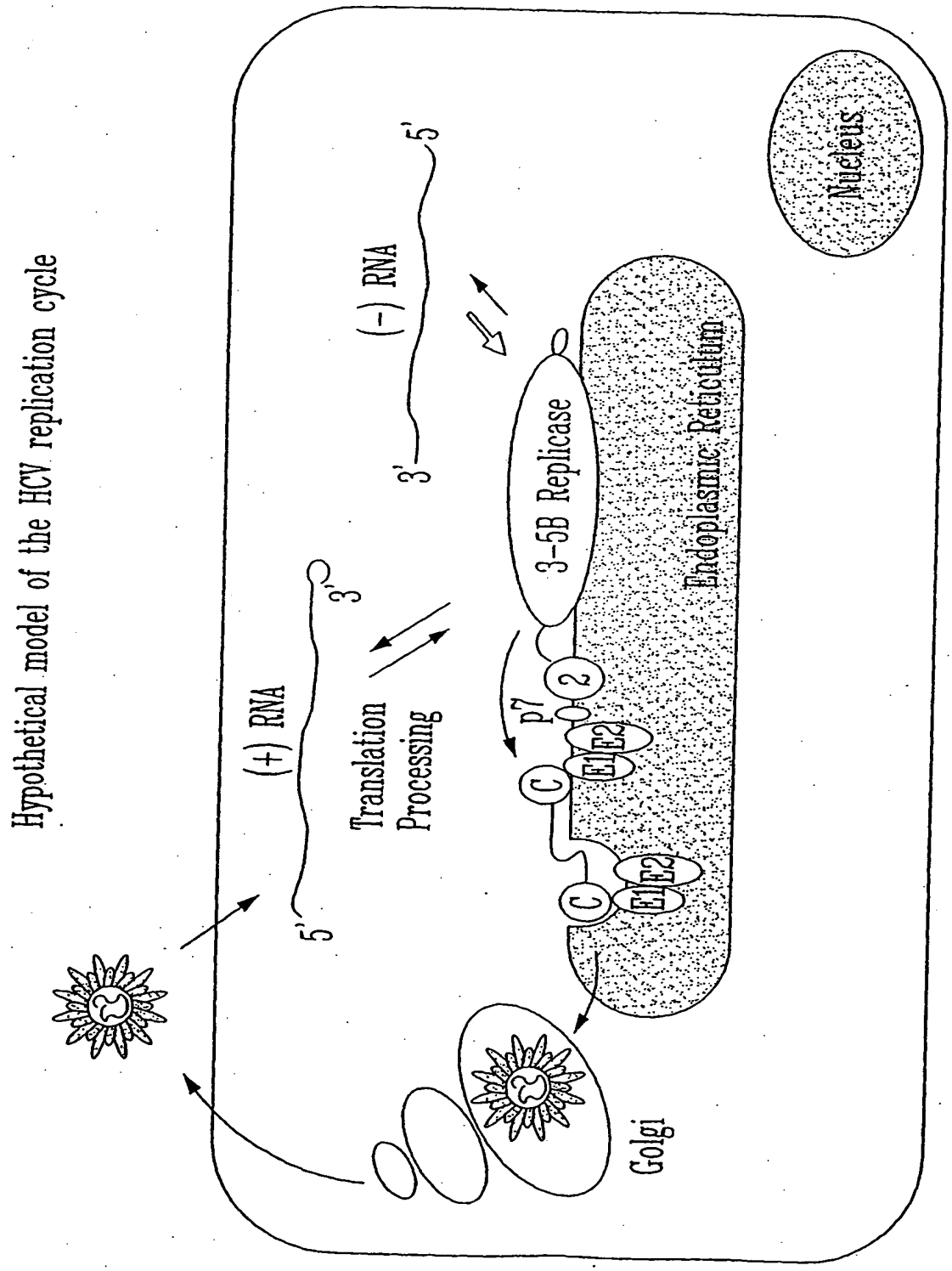

Hypothetical model of the HCV replication cycle
(+) RNA
5'
3'
Translation
Processing
(-) RNA
3'
5'
3-5B Replicase
p7
C
E1
E2
Endoplasmic Reticulum
Nucleus
Golgi

FIG. 2

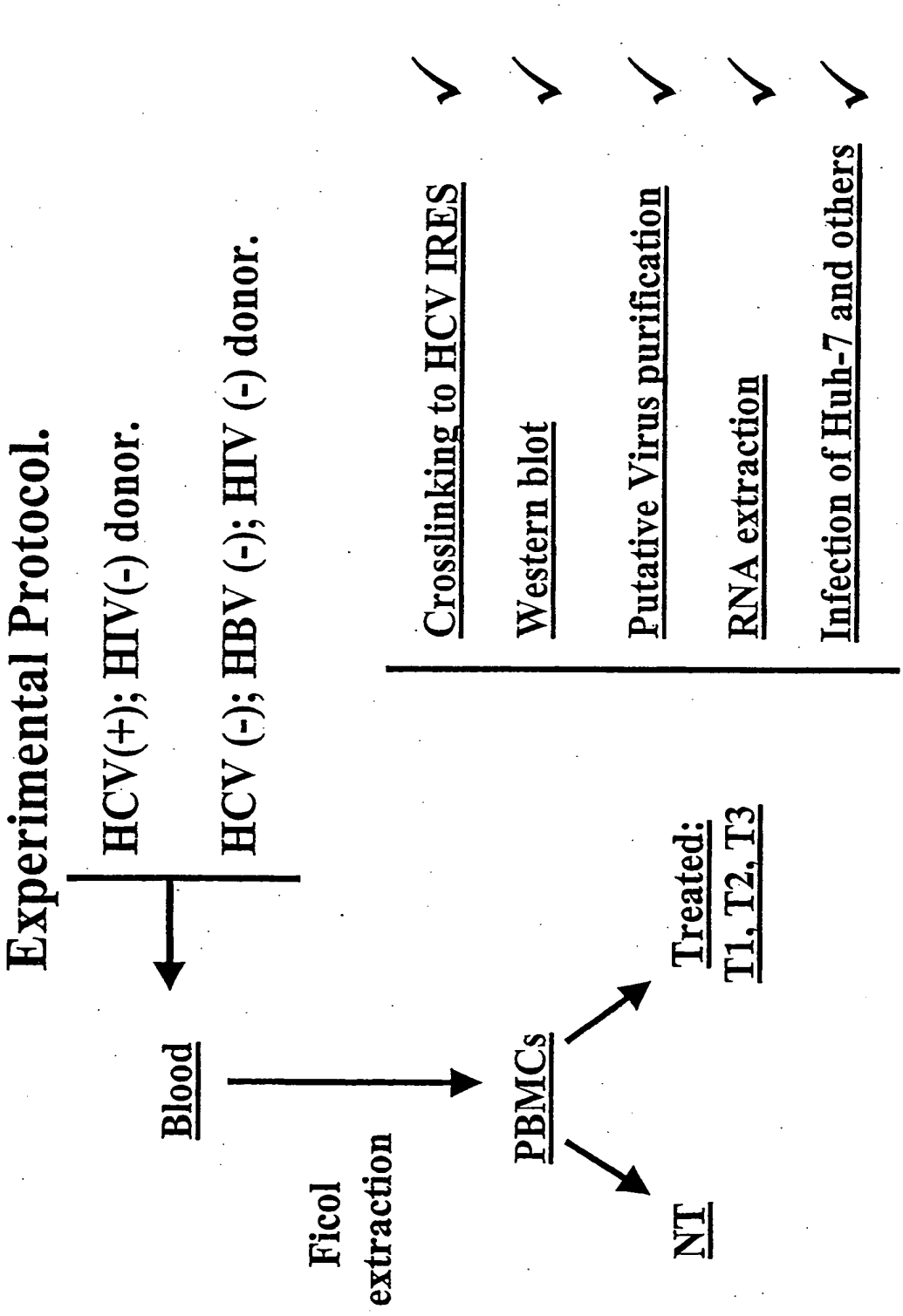

Experimental Protocol.
HCV(+); HIV(-) donor.
HCV (-); HBV (-); HIV (-) donor.
Blood
Ficol extraction
PBMCs
NT
Treated: T1, T2, T3
Crosslinking to HCV IRES
Western blot
Putative Virus purification
RNA extraction
Infection of Huh-7 and others

Fig. 3

PBMC and PBLC purification from blood samples.

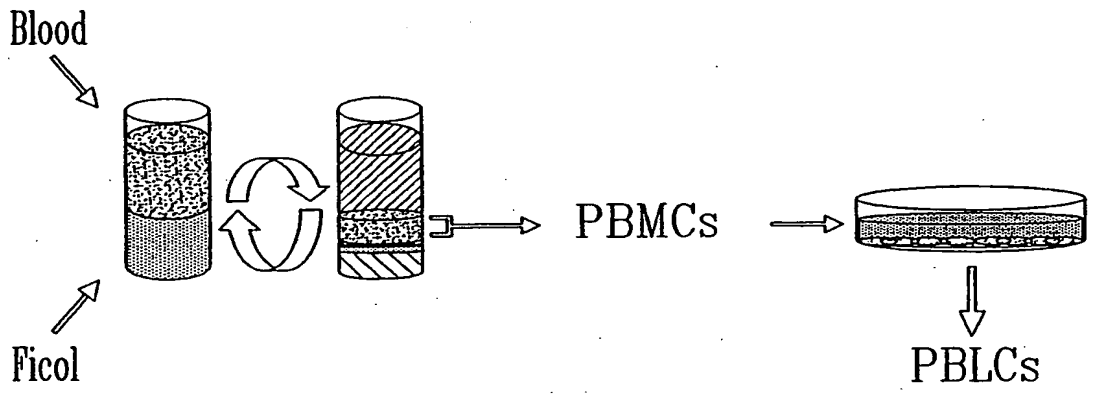


FIG_4

Detection of HCV NS3 and NS5 proteins in cell extracts from Treated PBMC from an HCV (+) patient.
[Boeringeranti-NS3 polyclonal antibody]

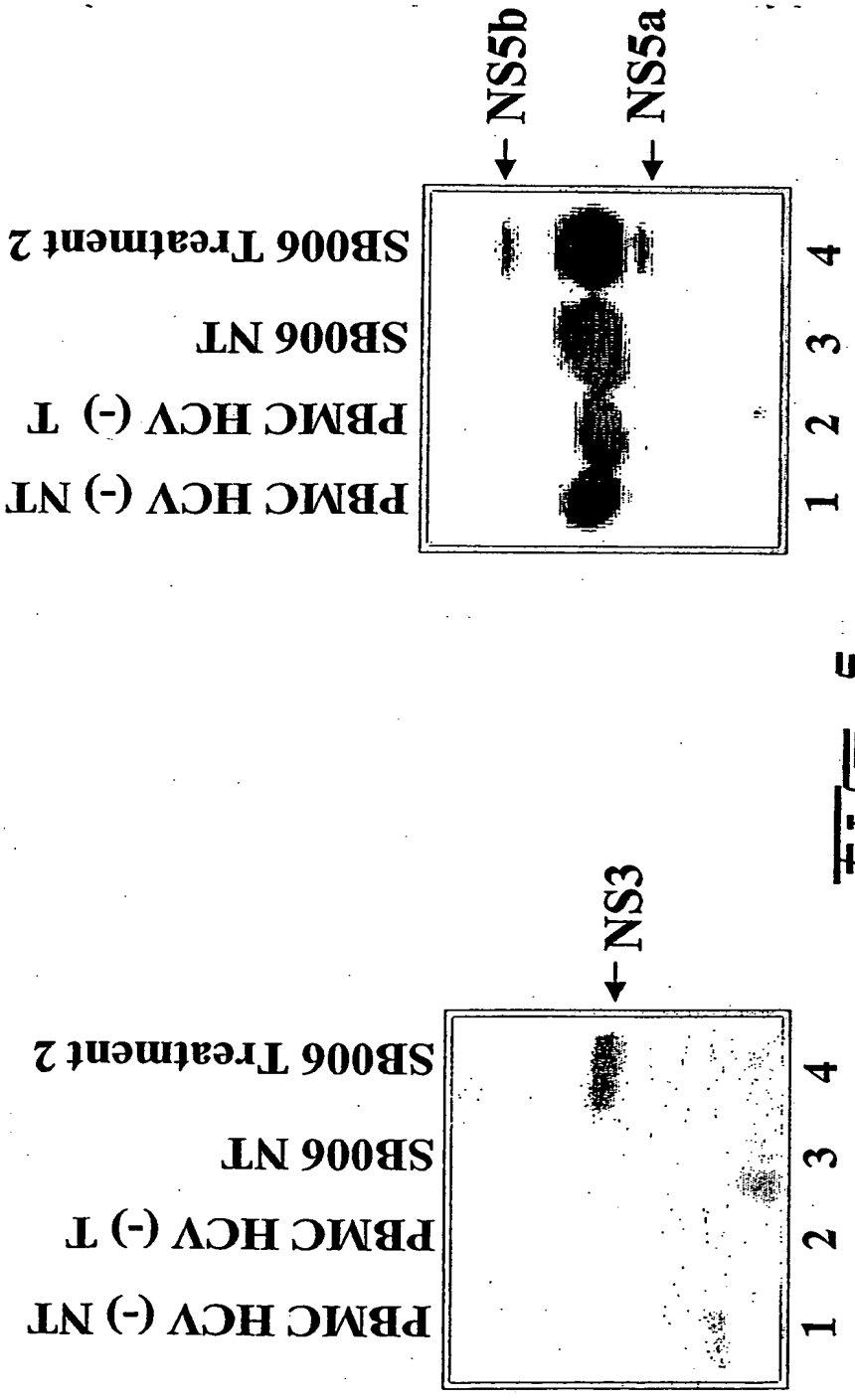


Fig 5

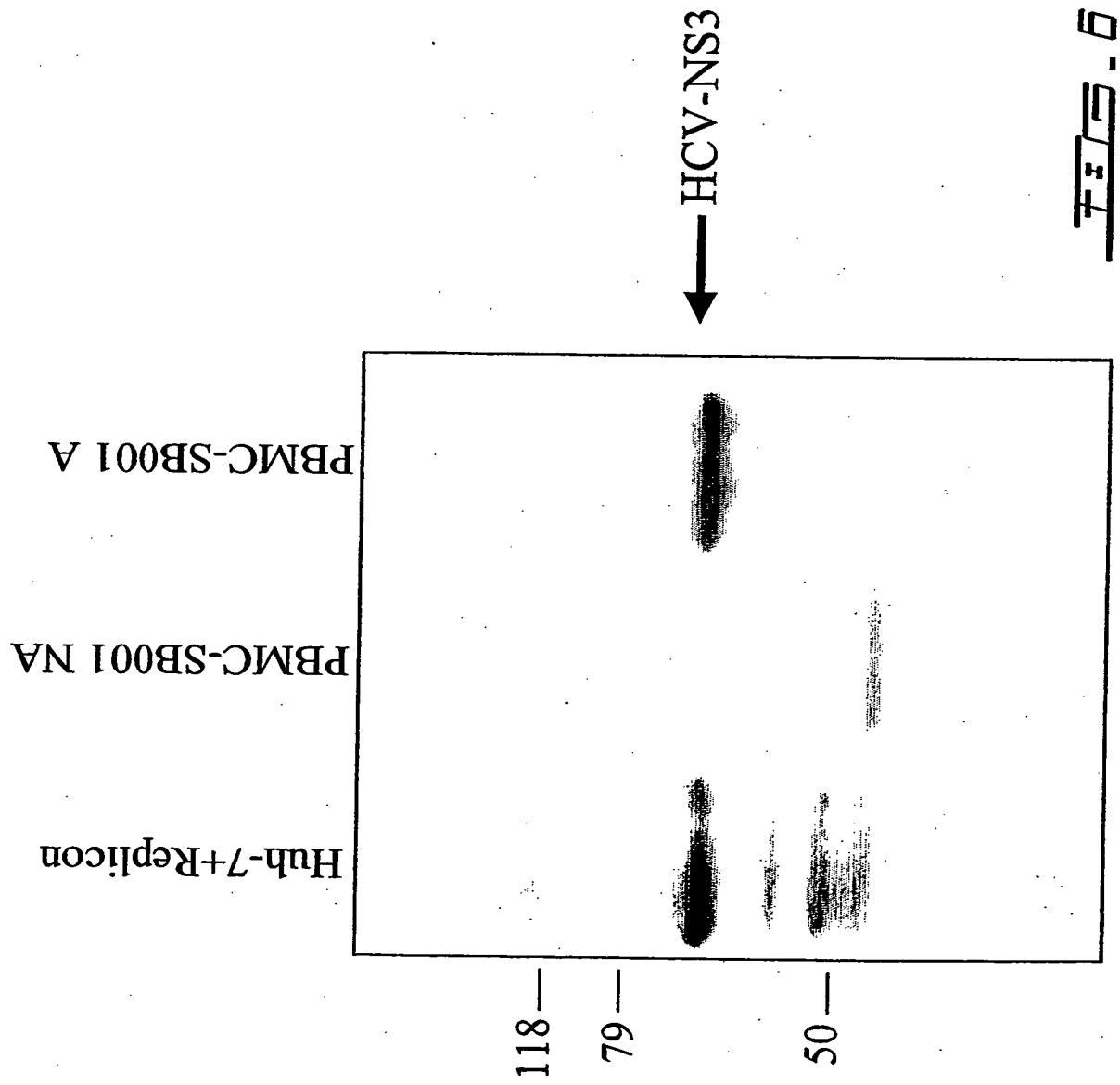


FIG. 6

Time course of HCV-NS3 detection: PBMCs From patient MLL-001

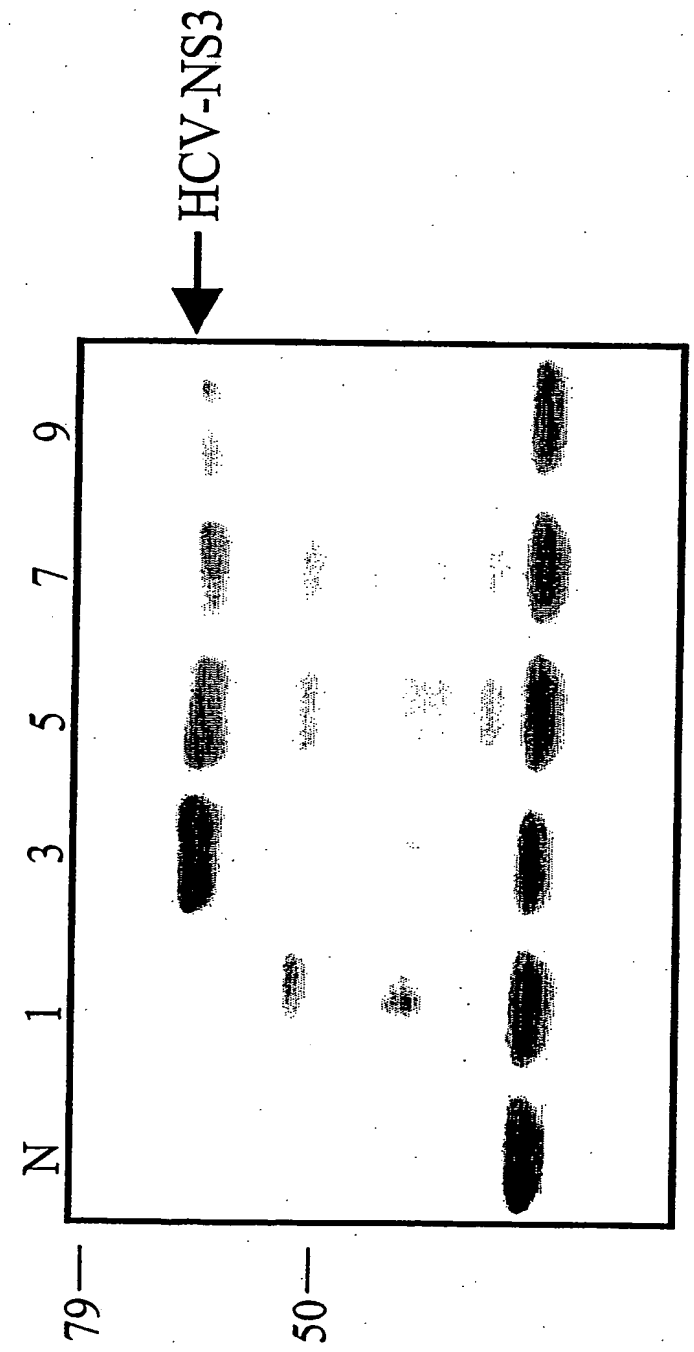


Fig. 7

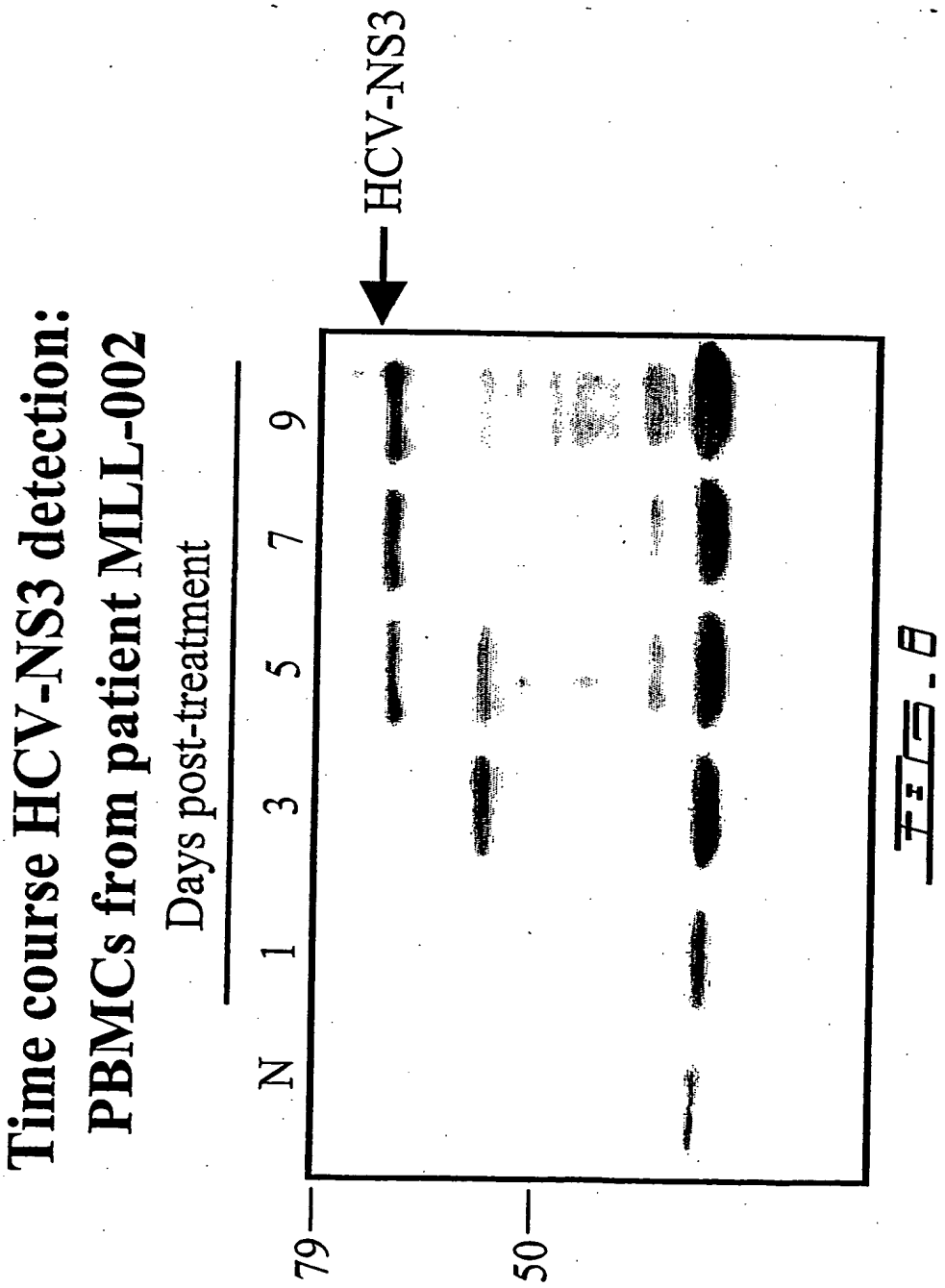

Time course HCV-NS3 detection:
PBMCs from patient MLL-002
Days post-treatment
N 1 3 5 7 9
HCV-NS3
79
50

FIG. 8

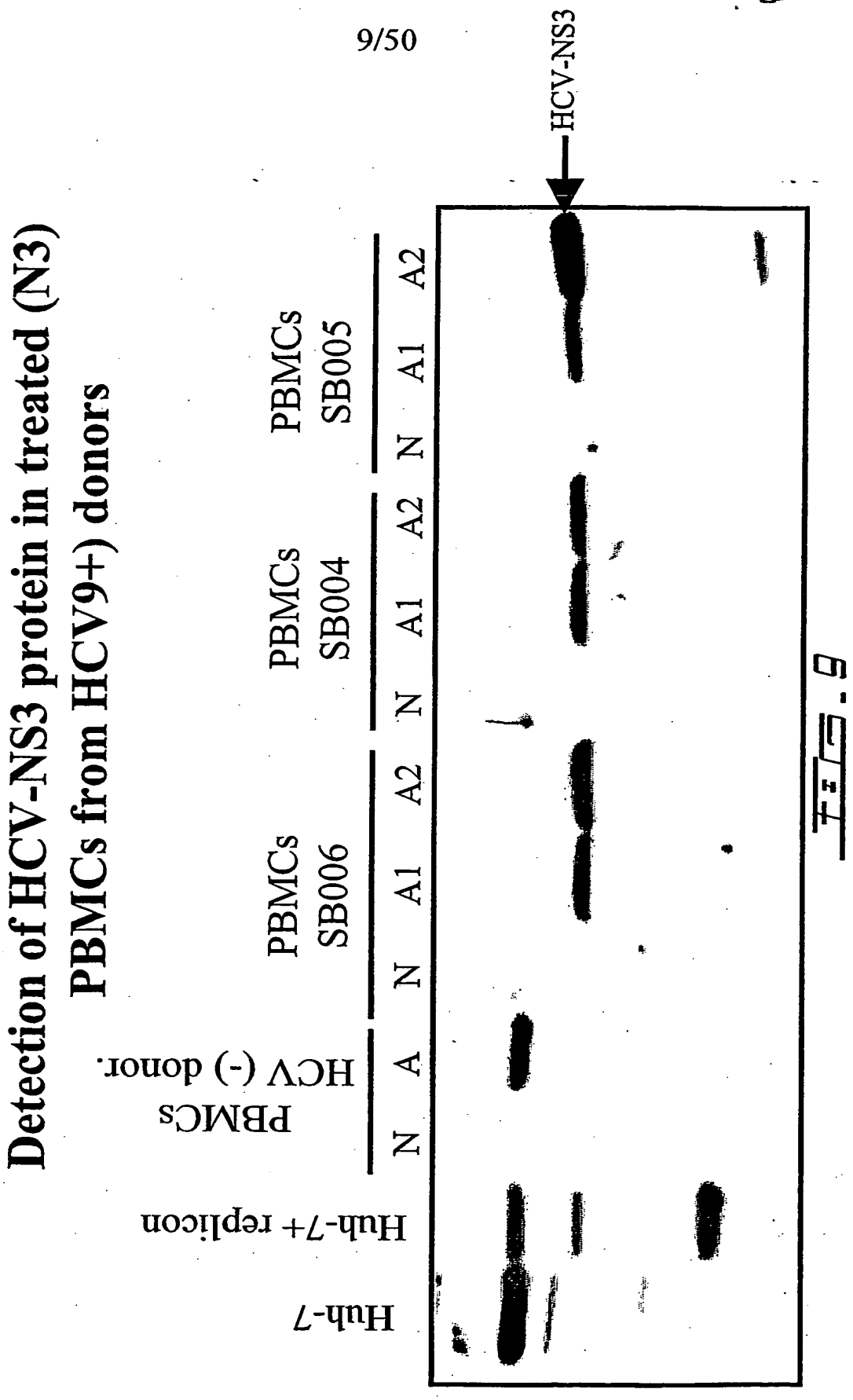
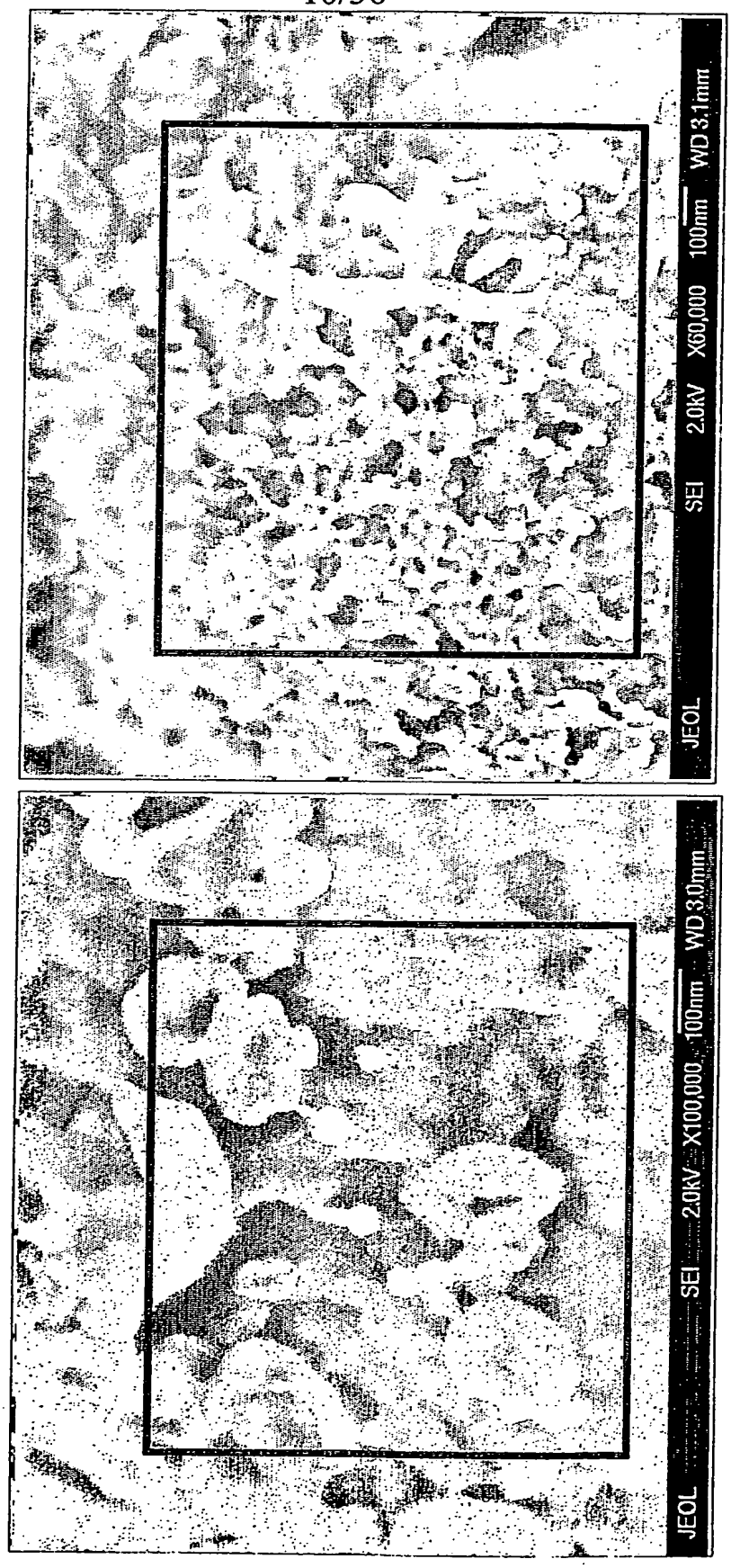


Fig. 9

10/523602

Detection of virus like particles by scanning electron microscopy



(-) Control

Fig. 10

Electron microscopy of Activated PBLCs;
Detection of virus like particles

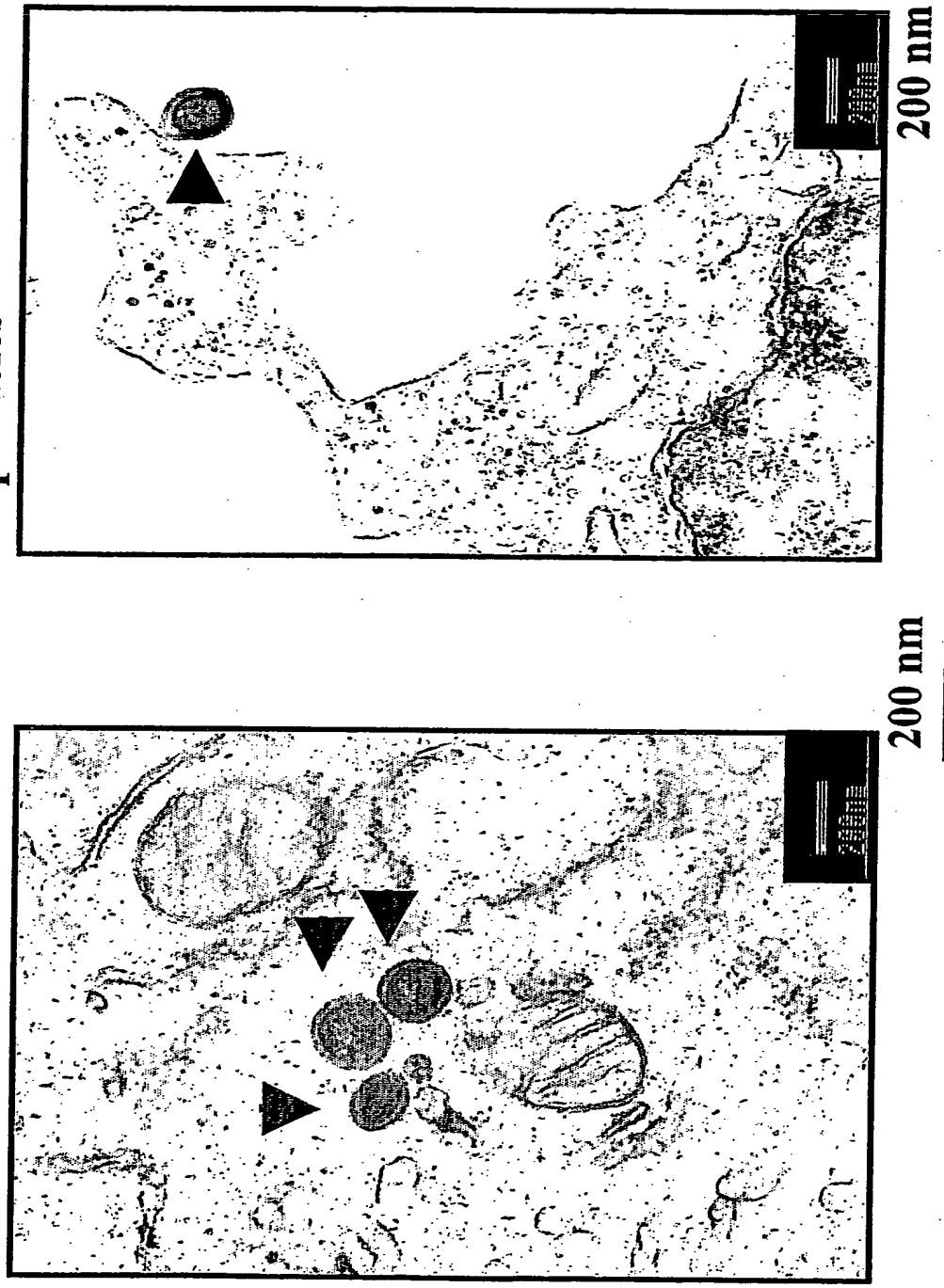


Fig. 11

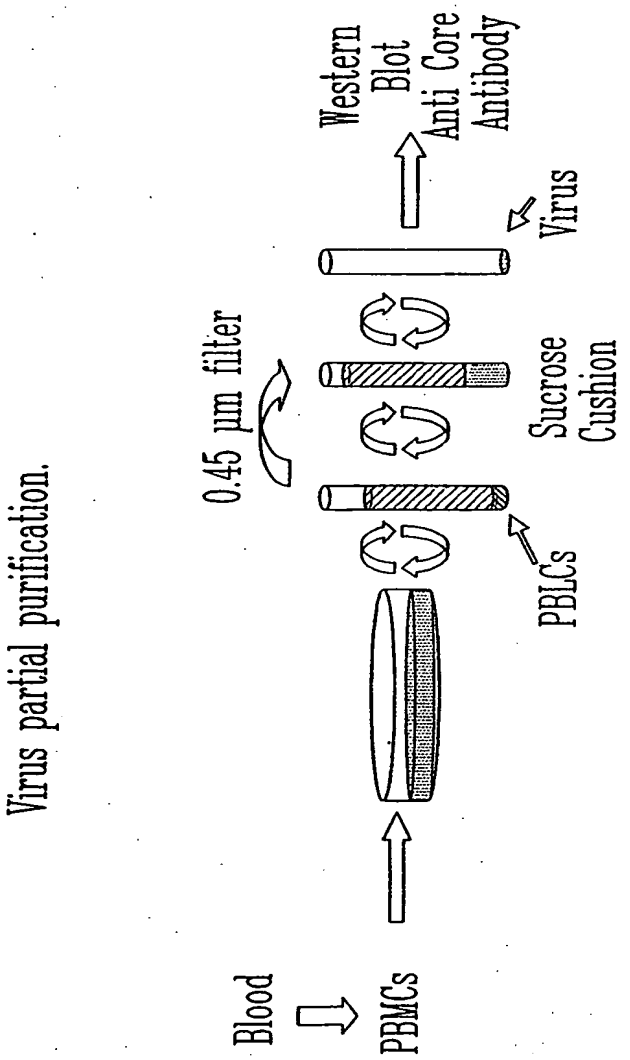


FIG. 12

Detection of HCV Core protein in supernatant of treated PBMC from an HCV (+) patient.
[Maine biotechnology anti-Core monoclonal antibody]

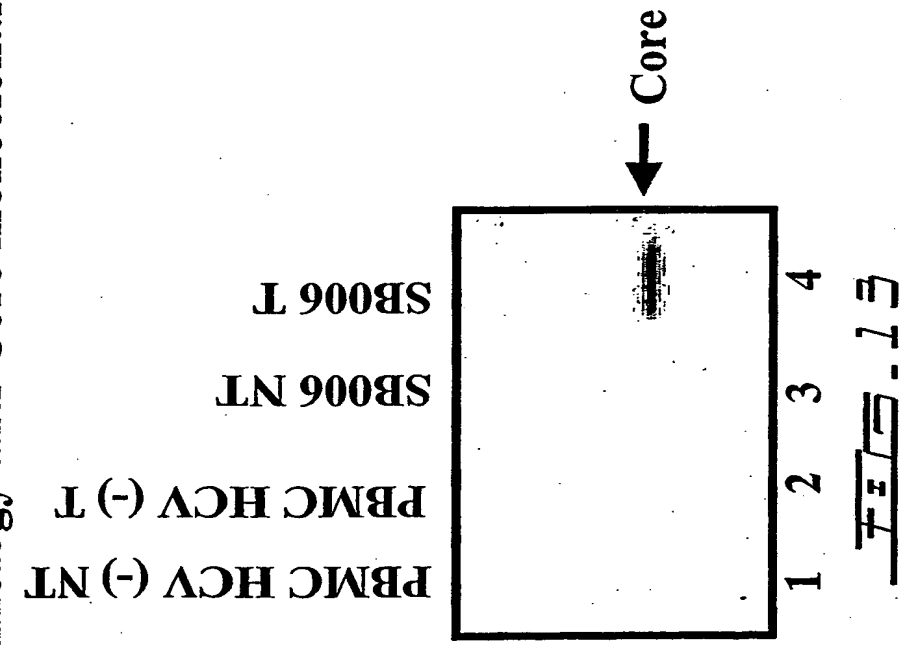


Fig. 13

RNA Quantification I (virus copies/ng total RNA)

Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant
After 4 days		
SB004 NT	$2x10^3$	No
SB004 T	$2x10^3$	Yes
SB006 NT	$1.8 x10^3$	No
SB006 T	$2x10^2$	Yes
After 20 days		
SB004	0.00	
SB006	0.00	

FIG. 14

10/523602

Infection assay; co-culture

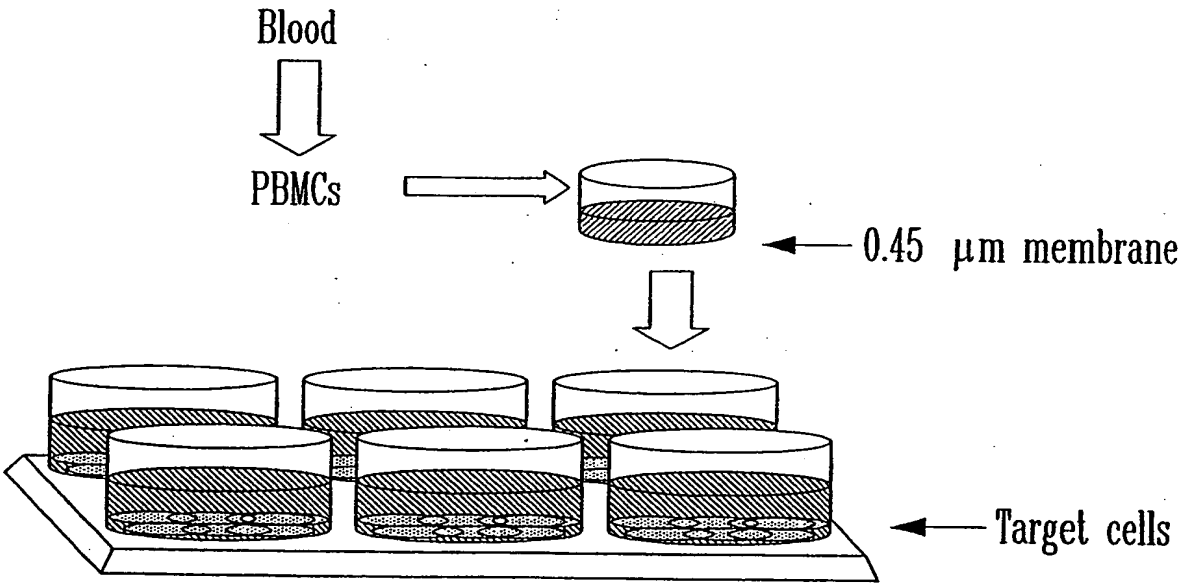


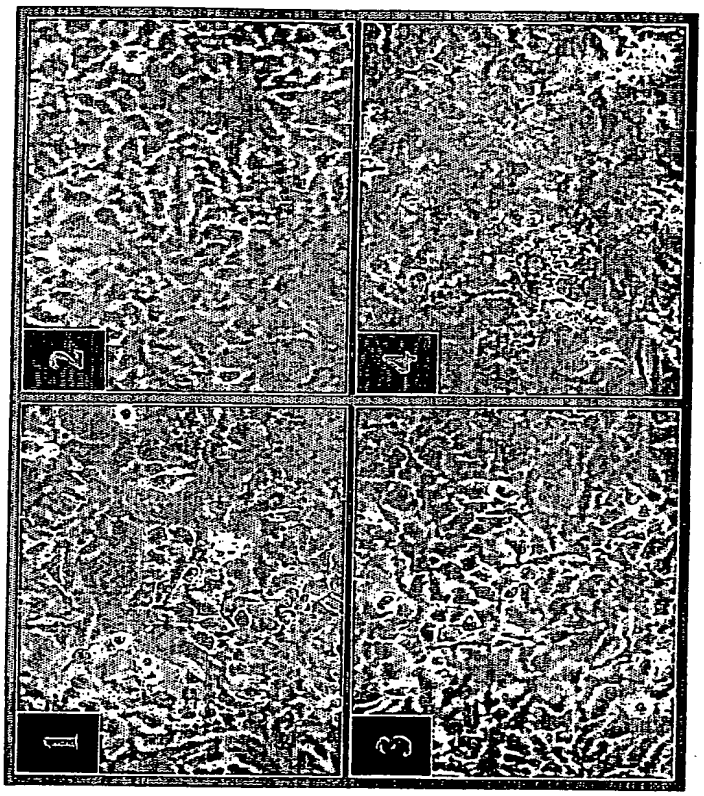
Fig. 15

Infection of MT-4 cells
RNA Quantification II (virus copies/ng total RNA)

Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant	HCV RNA In MT-4
After 10 days			
SB001 NT	13	No	0.00
SB001 T	12	Yes	1600
After 20 days			
SB001	0.00		0.00
SB001	0.00		0.00

FIG. 16

Co-culture of Huh-7 and HCV (-) PBMCs.



1- Huh-7
2- Huh-7 + PBMCs HCV (-) NT
3- Huh-7 + Treatment
4- Huh-7 + PBMCs HCV (-) T

Fig. 17

Co-culture of Huh-7 and HCV (+) PBMS° Cs (SB006).

1. Huh-7
2-3. Huh-7 + PBMCs HCV (+) NT
4. Huh-7 + Treatment
5-6. Huh-7 + PBMCs HCV (+) T

Fig. 18

PHA Activation of PBMCs from patient SB004;
HCV is not in T cells

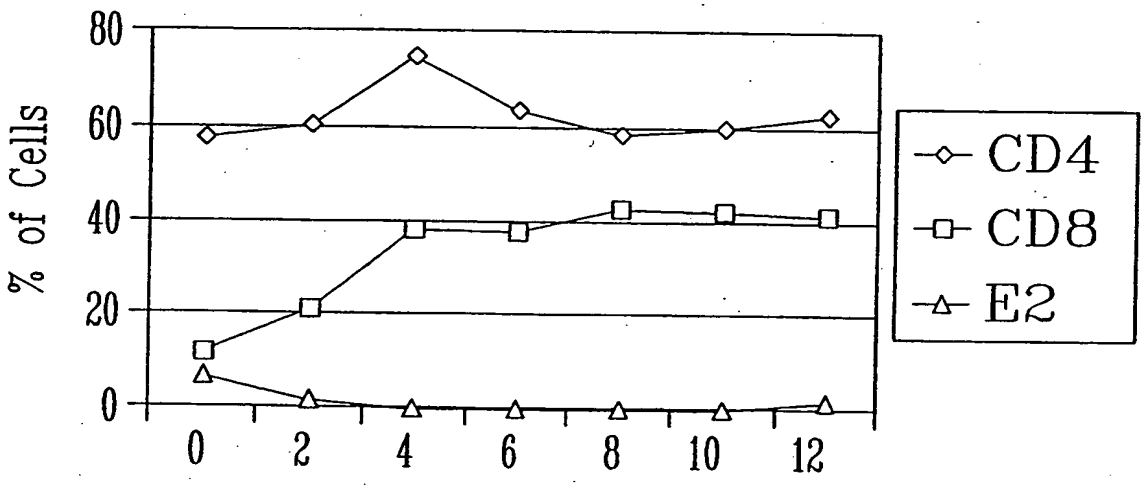


Fig. 19

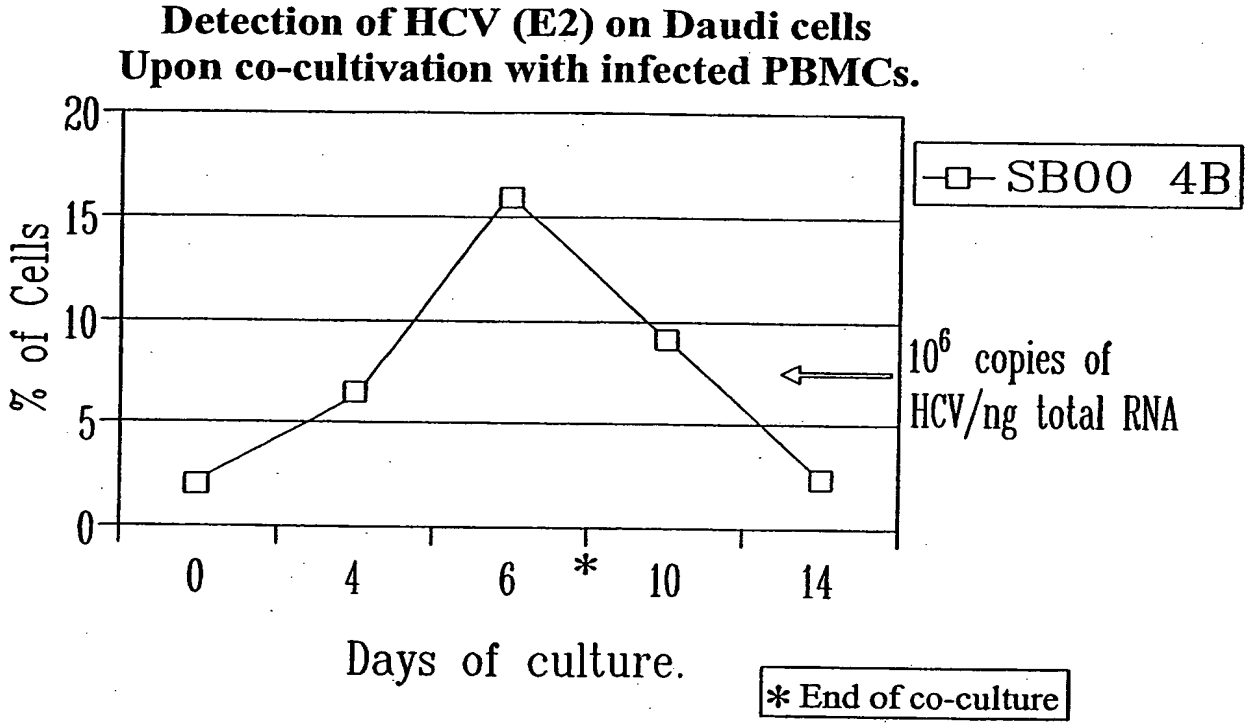

Detection of HCV (E2) on Daudi cells
Upon co-cultivation with infected PBMCs.
% of Cells
20
15
10
5
0
0
4
6
*
10
14
Days of culture.
SB00 4B
10^6 copies of HCV/ng total RNA
* End of co-culture

Fig. 20

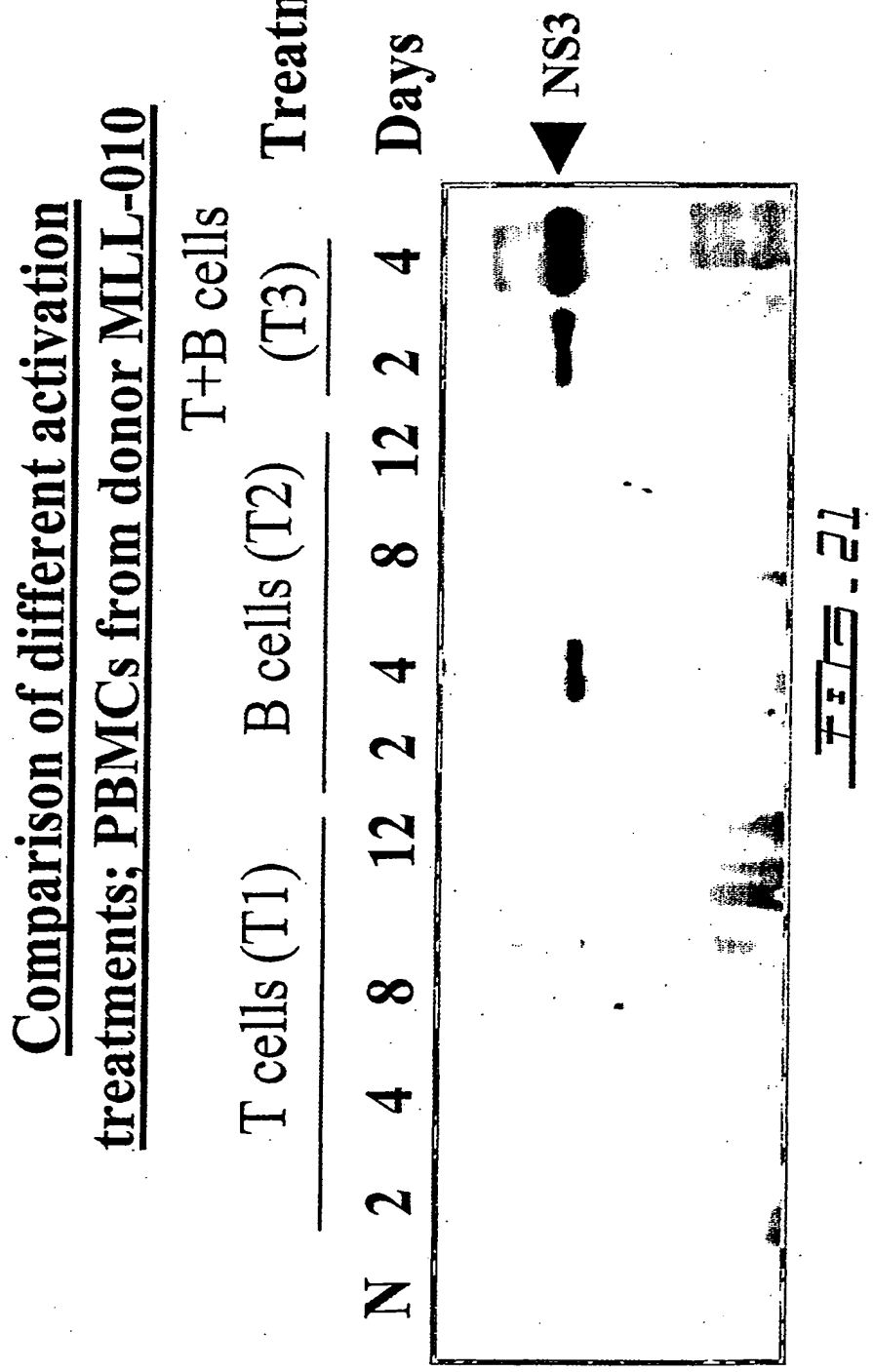

Comparison of different activation
treatments; PBMCs from donor MLL-010
T+B cells
T cells (T1) B cells (T2) (T3)
Treatment
N 2 4 8 12 2 4 8 12 2 4
Days
NS3

Fig. 21

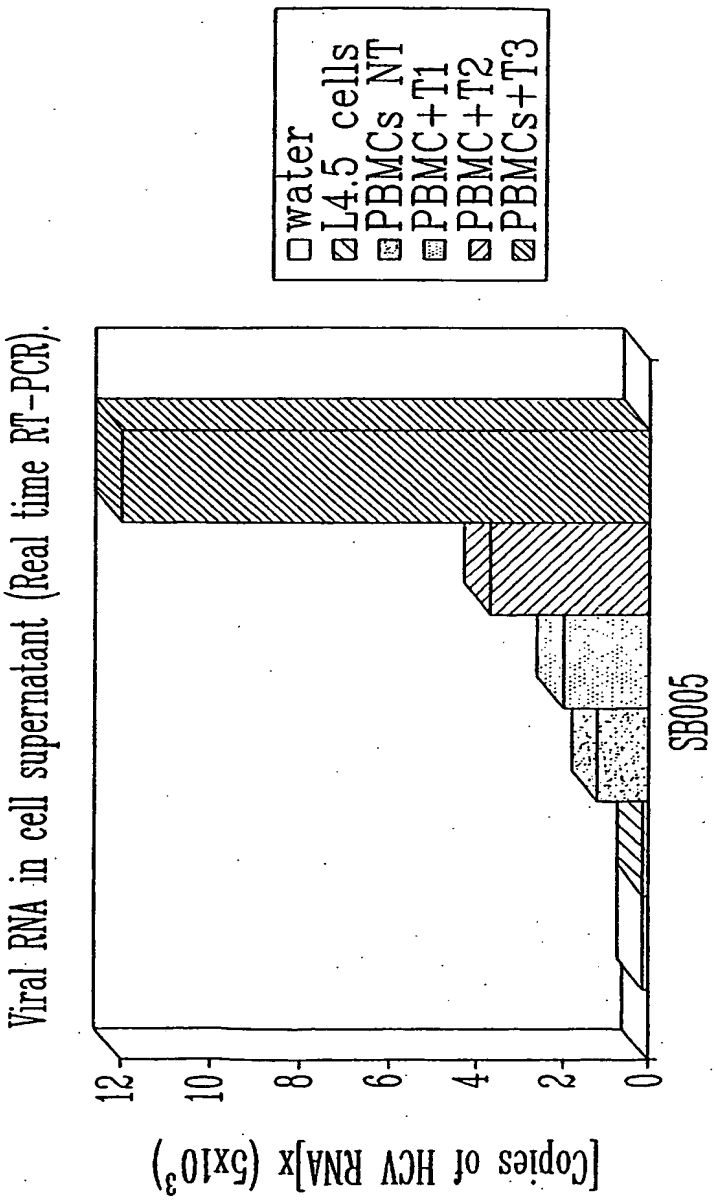


Fig. 22

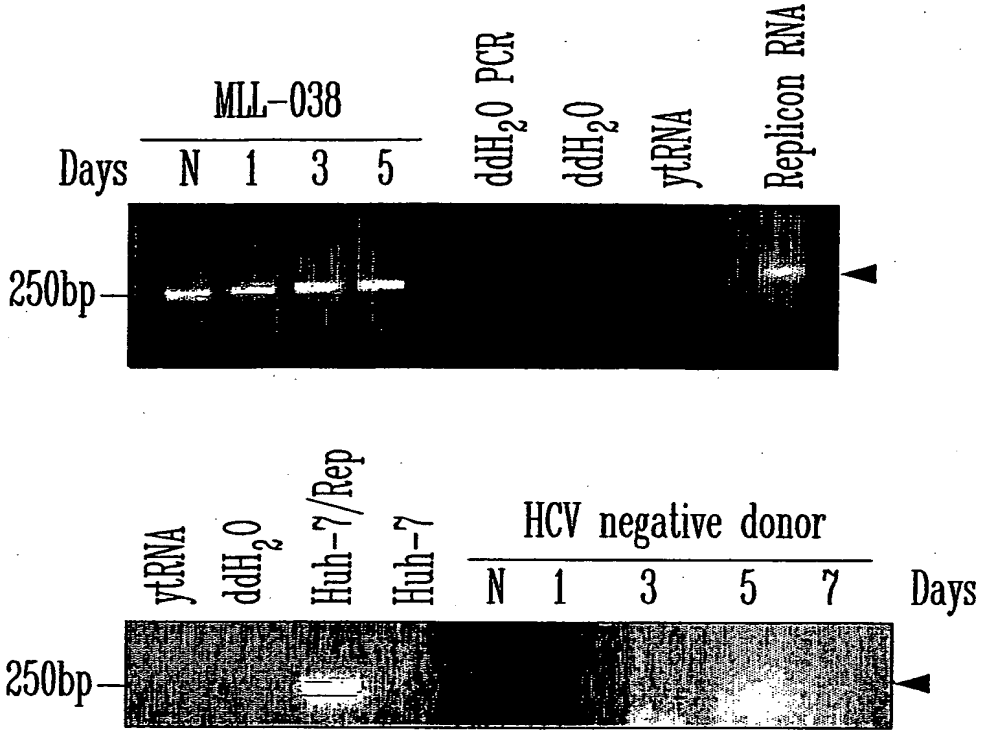


FIG_23A

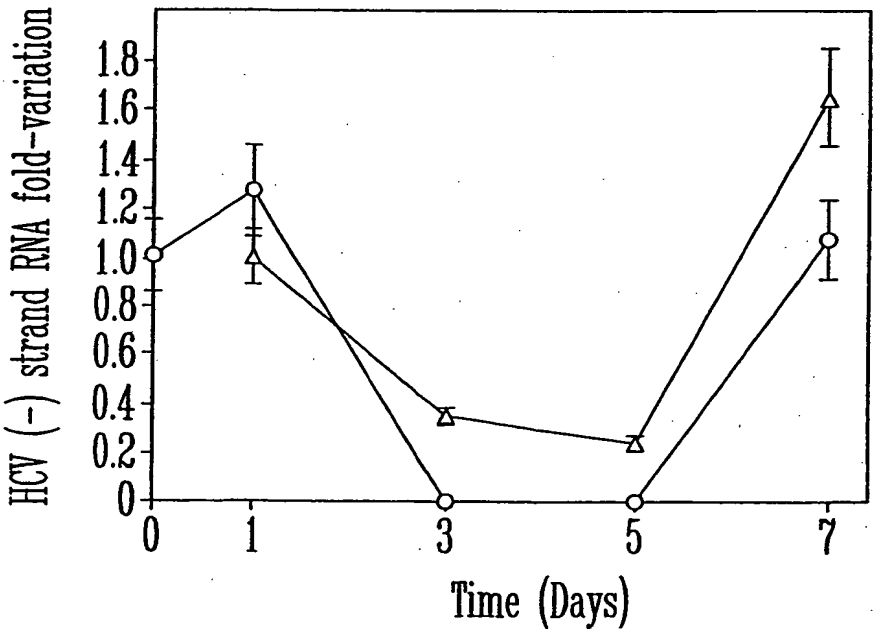


FIG_23B

AU/523602

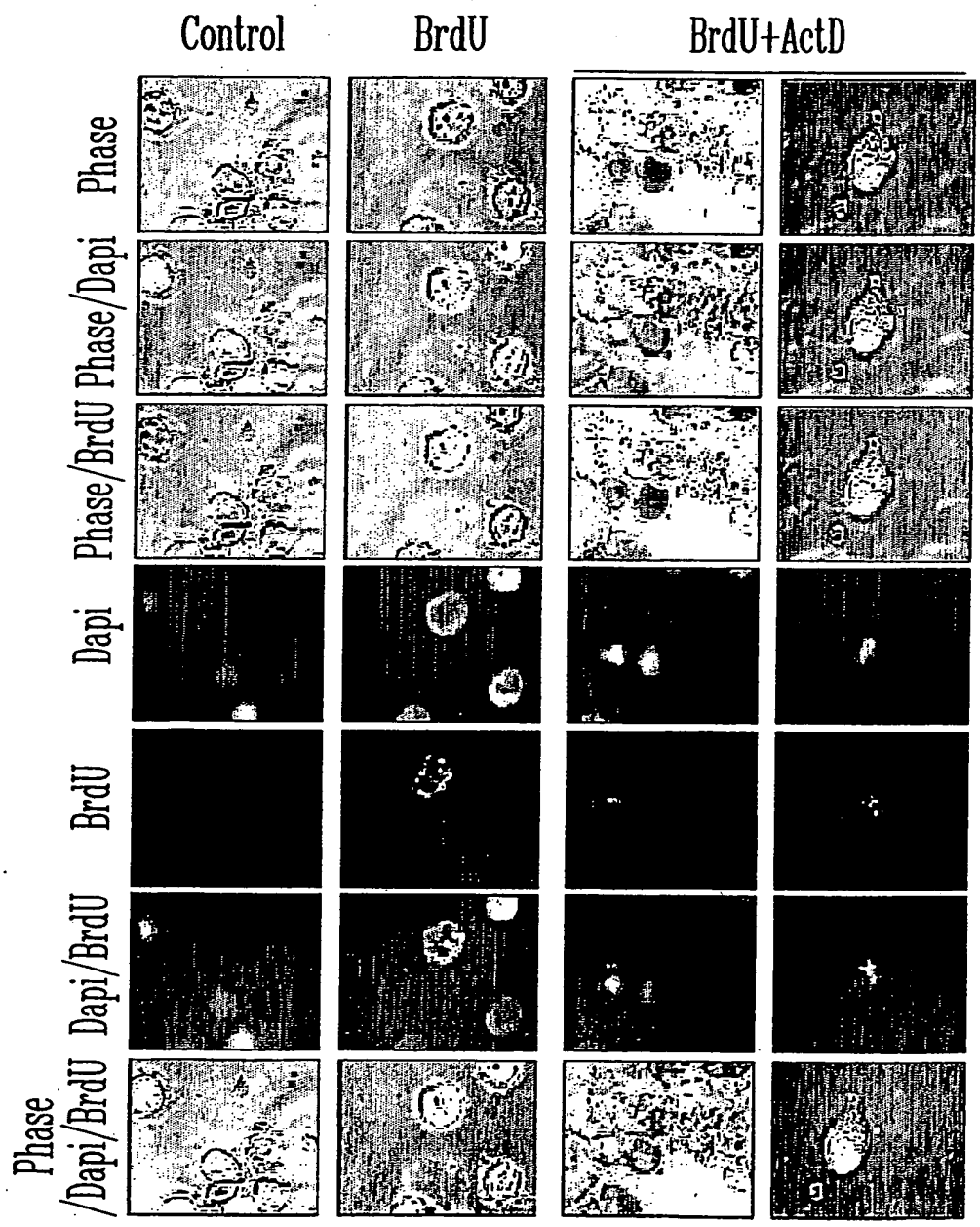


FIG. 23C

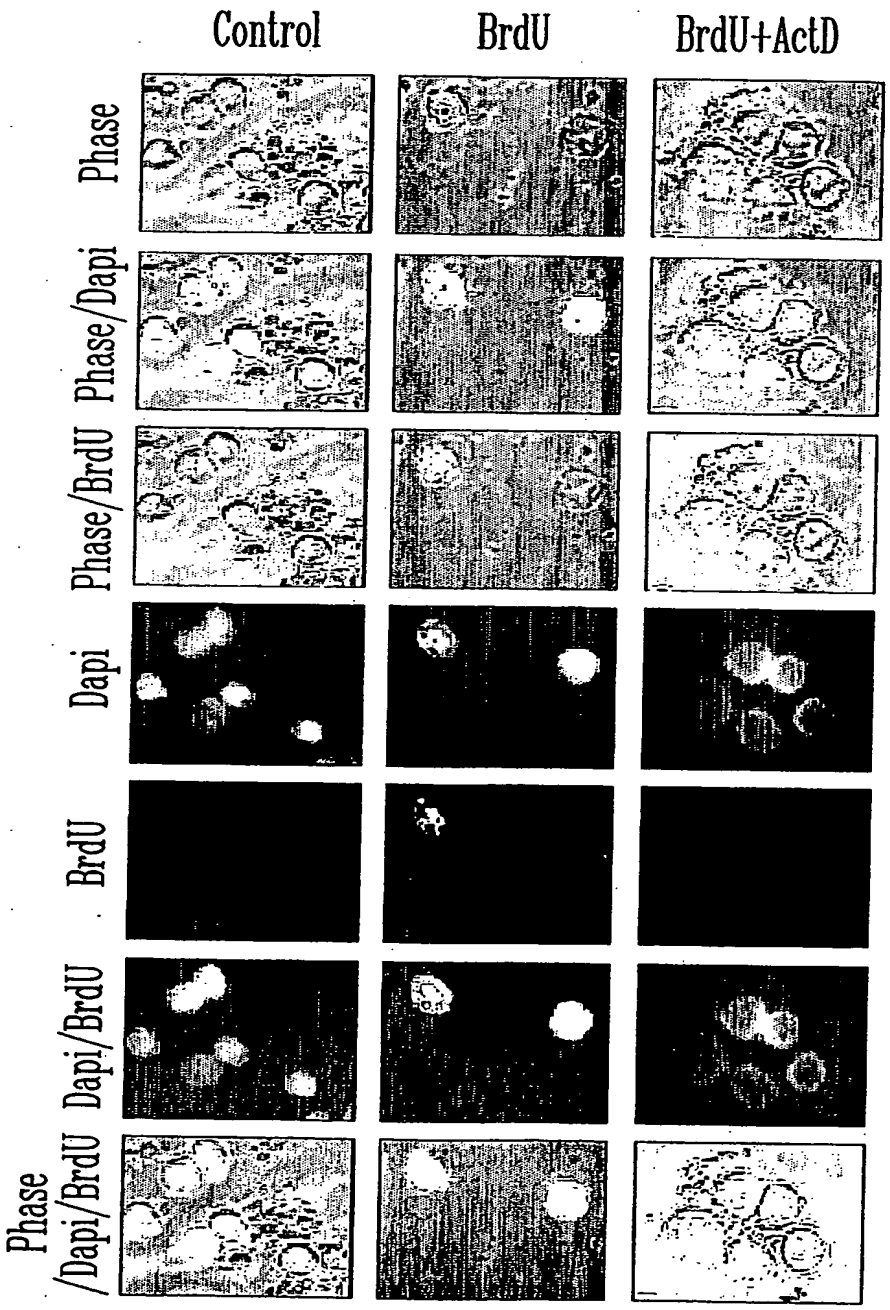

Control
BrdU
BrdU+ActD
Phase
Phase/Dapi
Phase/BrdU
Dapi
BrdU
Dapi/BrdU
Phase /Dapi/BrdU

FIG-23D

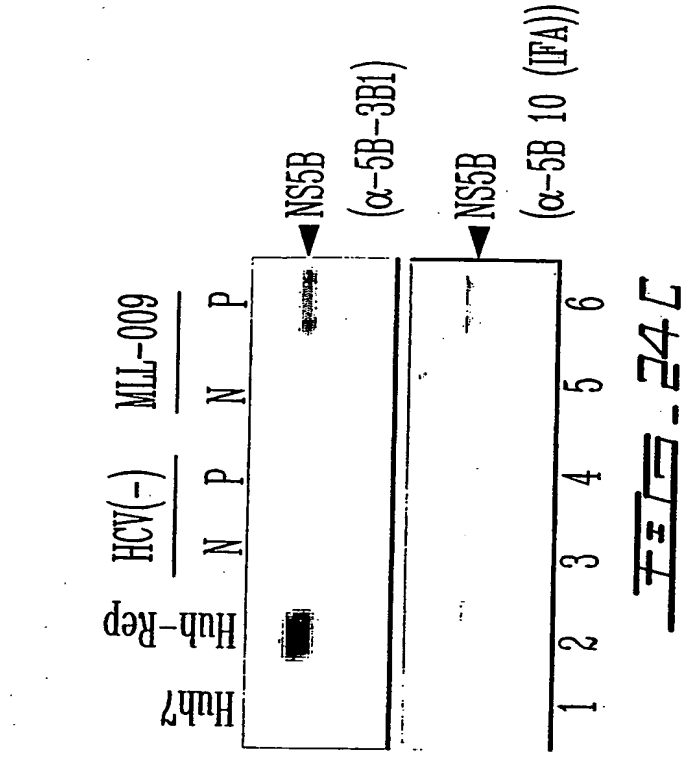


FIG. 24C

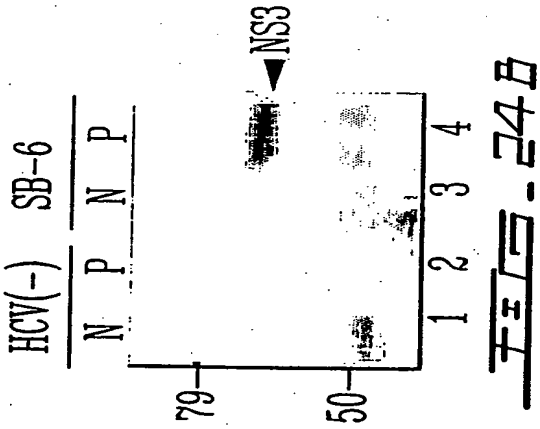


FIG. 24B

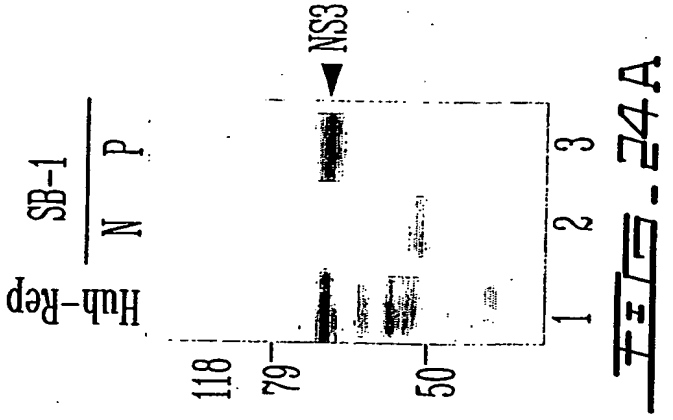


FIG. 24A

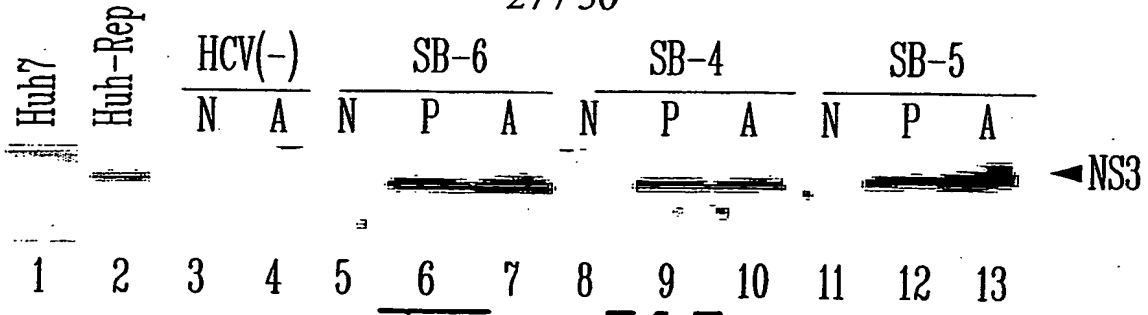


FIG_24D

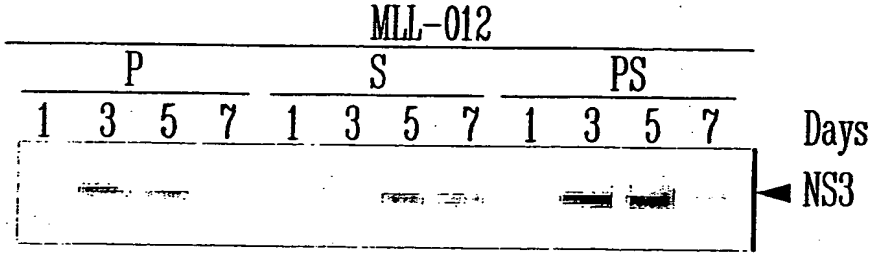


FIG_24E

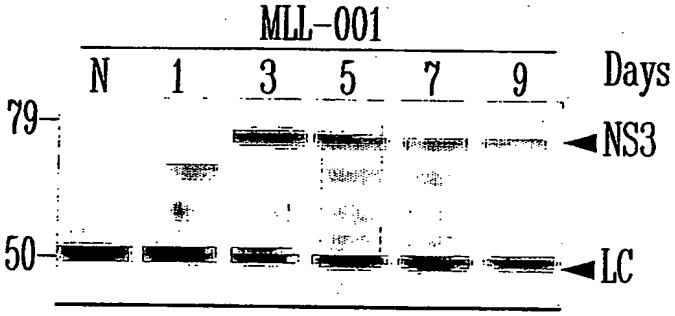


FIG_24F

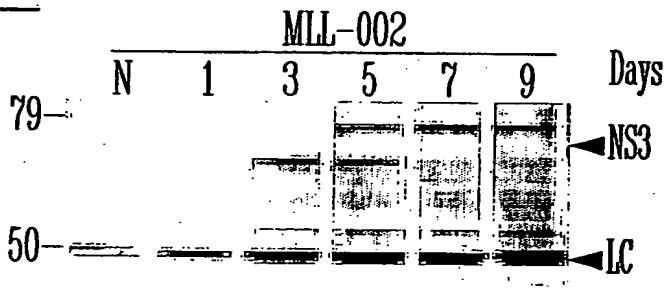


FIG_24G

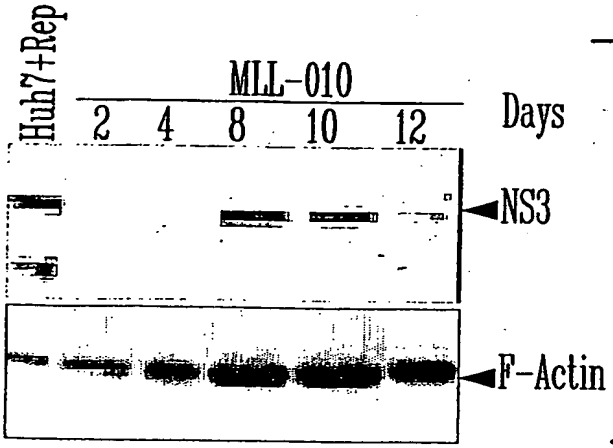


FIG_24H

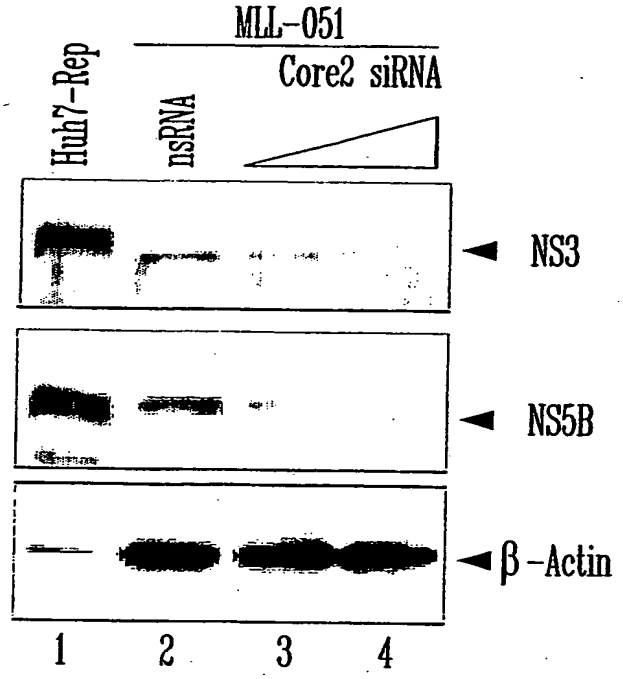


FIG_24I

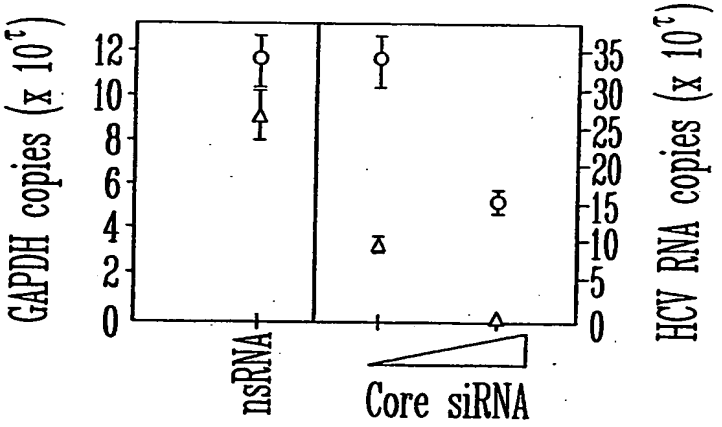


FIG_24J

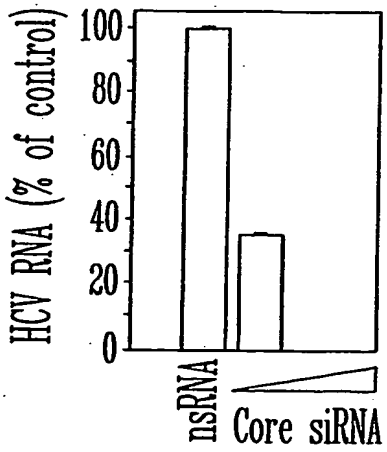


FIG_24K

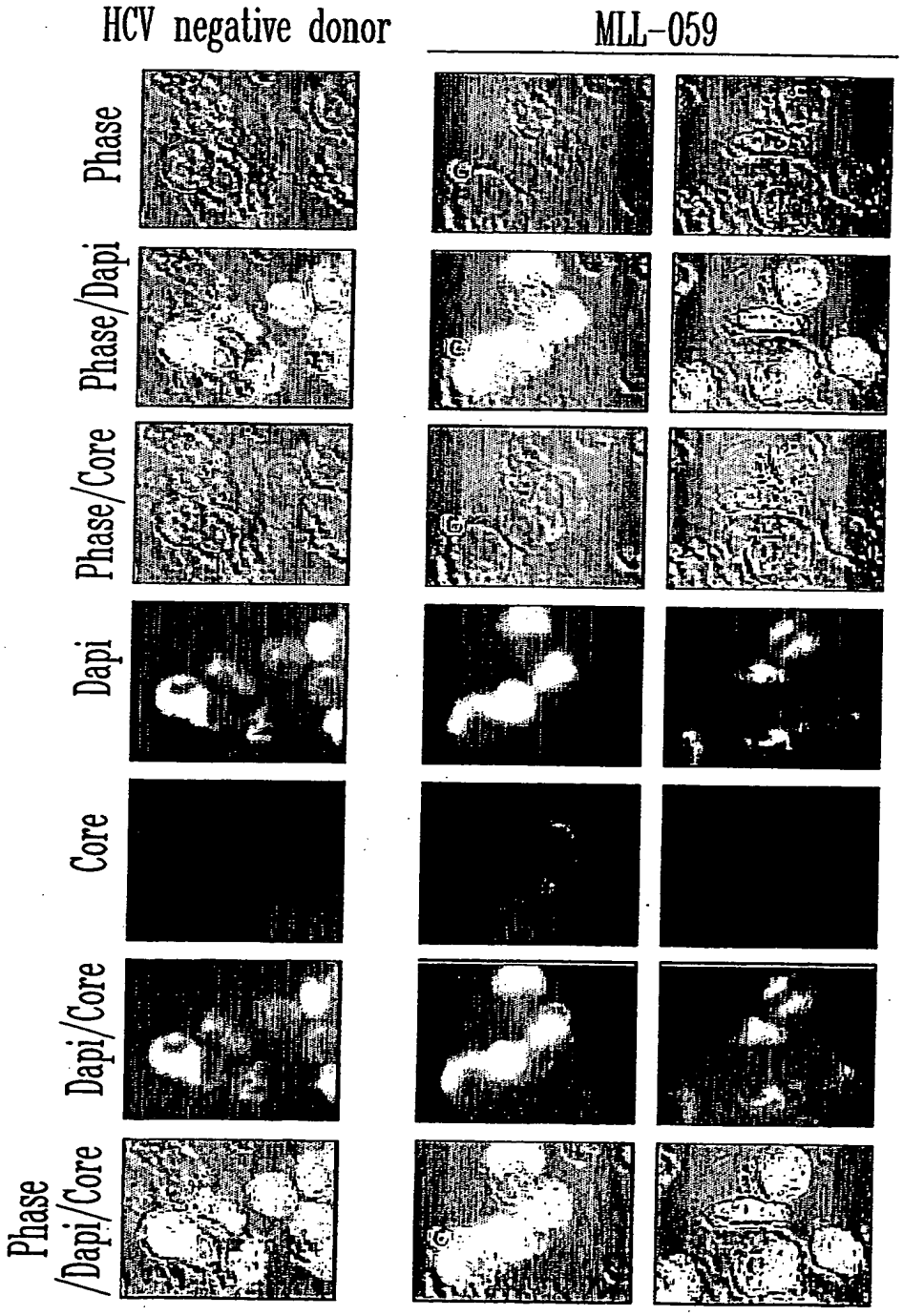

HCV negative donor
MLL-059
Phase
Phase/Dapi
Phase/Core
Dapi
Core
Dapi/Core
Phase /Dapi/Core

Fig. 25

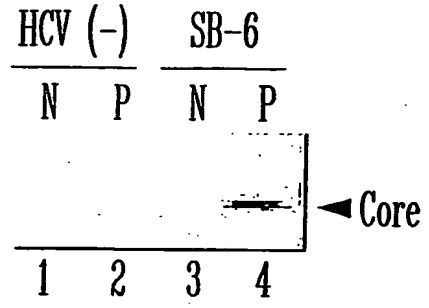


FIG_26A

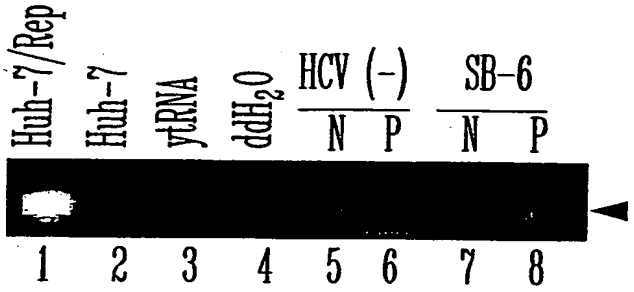


FIG_26B

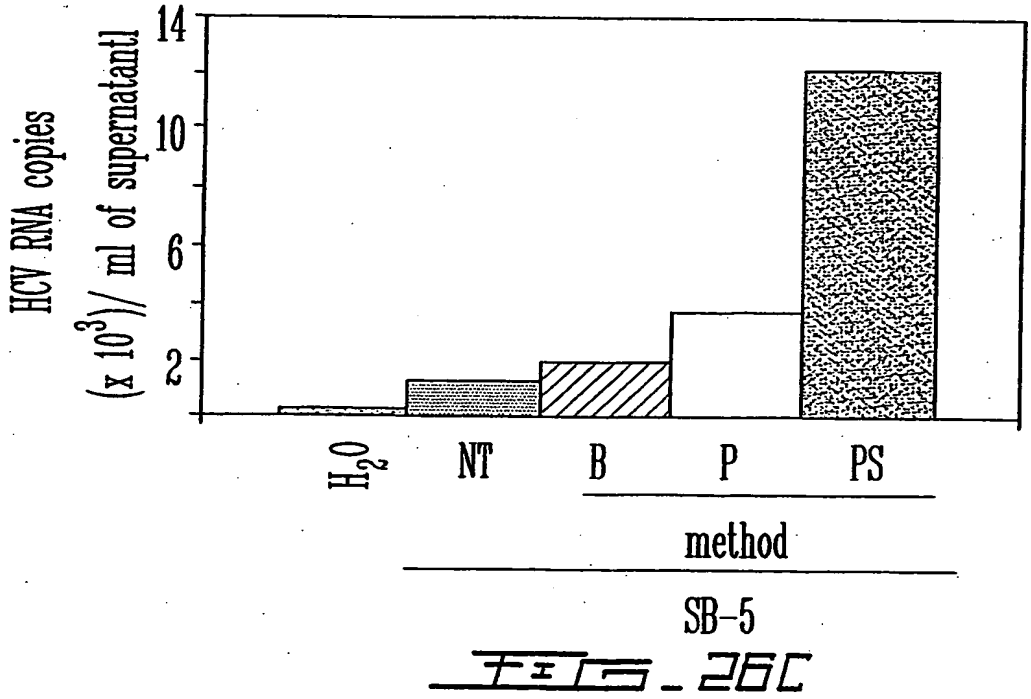


FIG_26C

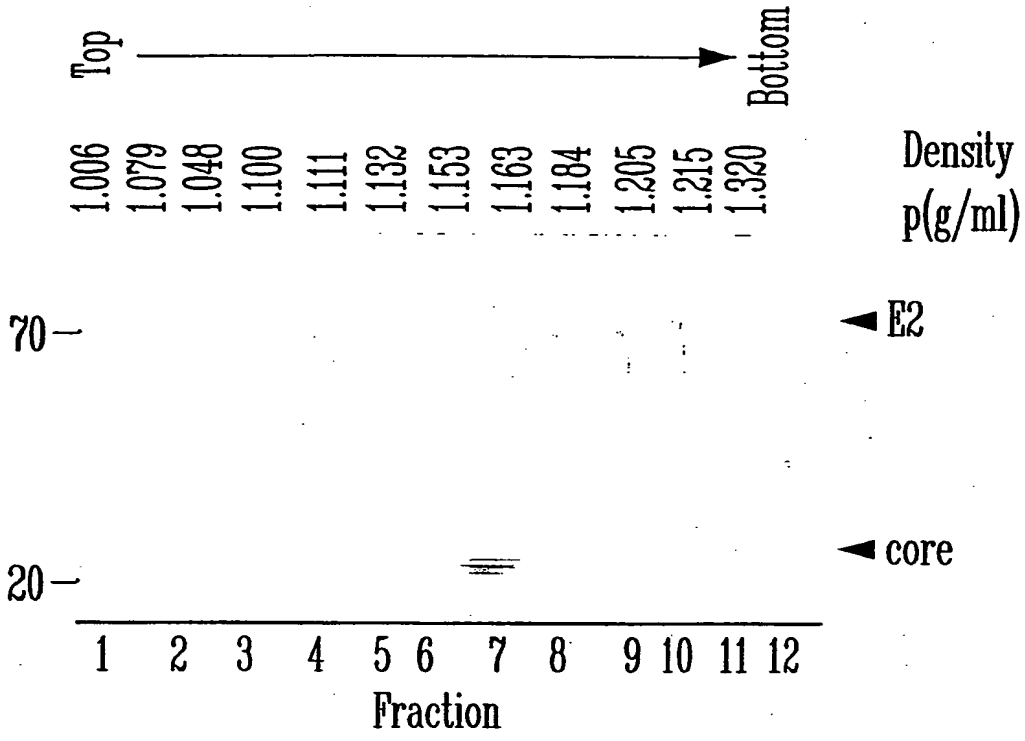


FIG_26D

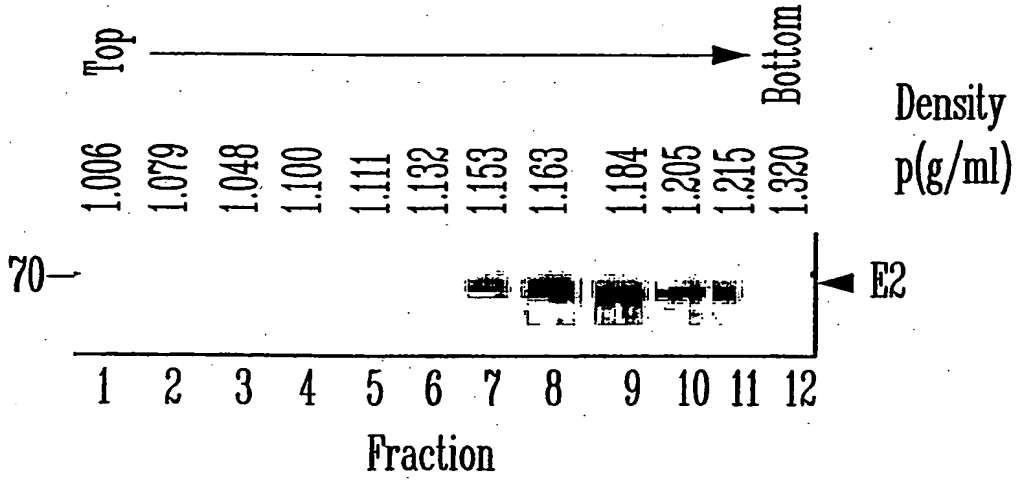


FIG_26E

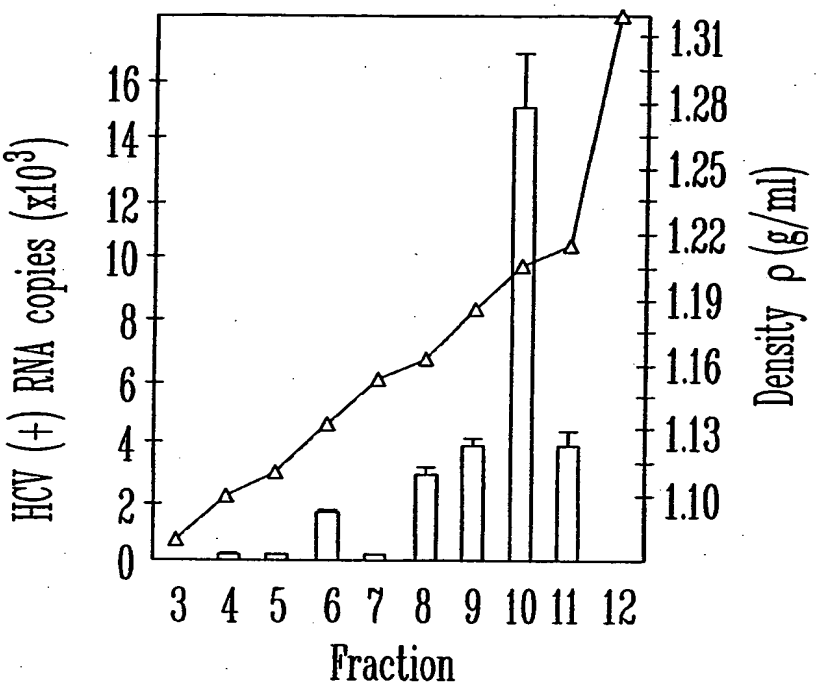


FIG_26F

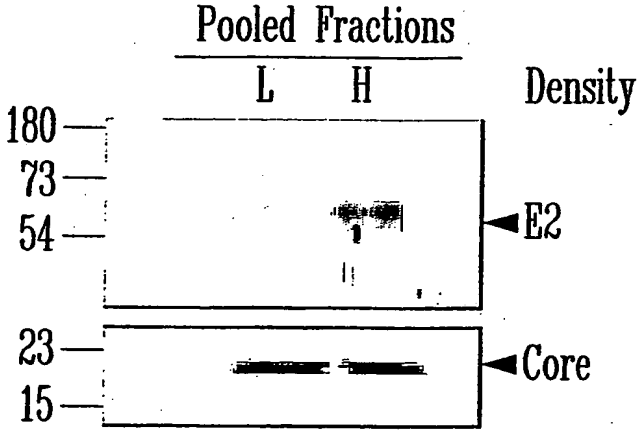


FIG_26G

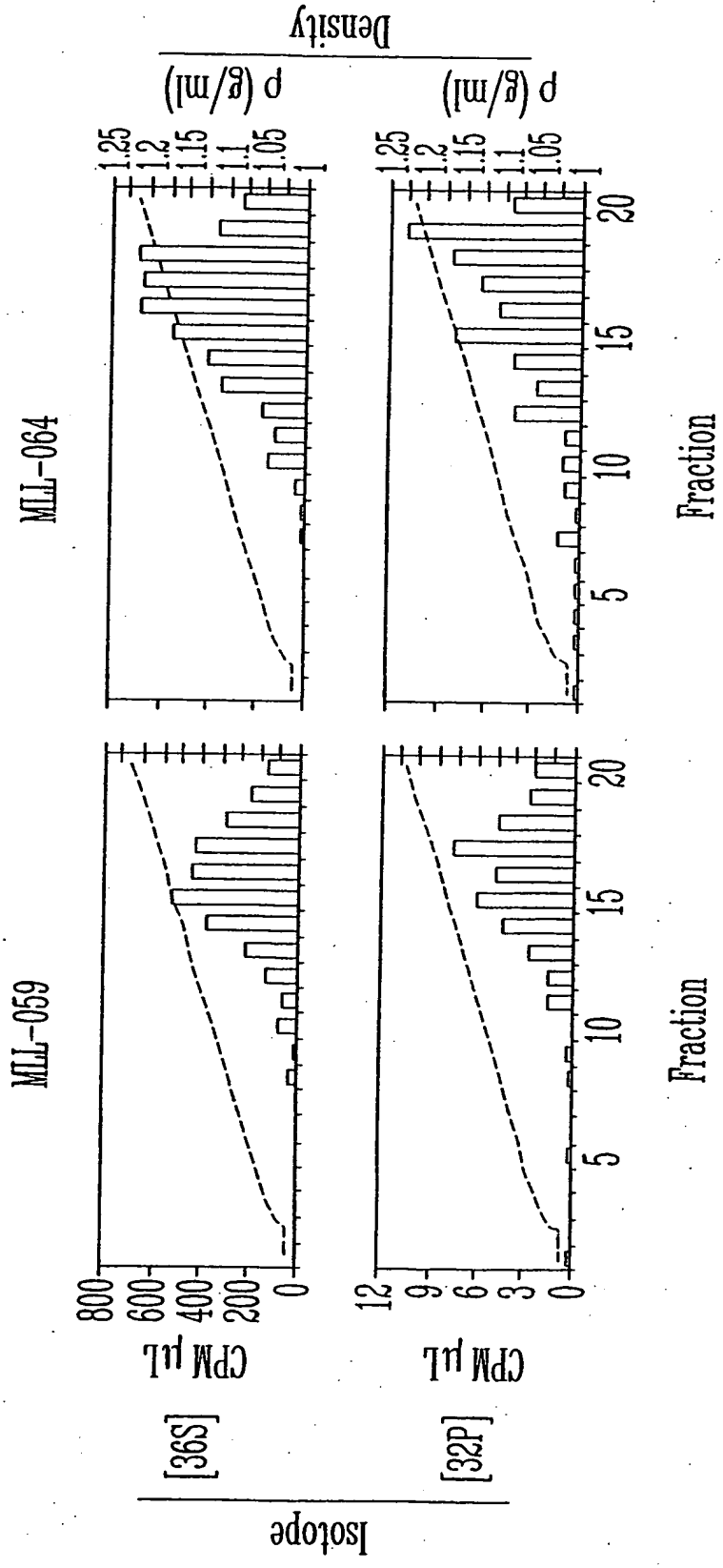

Density
ρ (g/ml)
ρ (g/ml)
1.25
1.2
1.15
1.1
1.05
1
MLL-064
MLL-059
Fraction
Fraction
0
5
10
15
20
0
3
6
9
12
0
200
400
600
800
CPM µL
CPM µL
[36S]
[32P]
Isotope

FIG. 26H

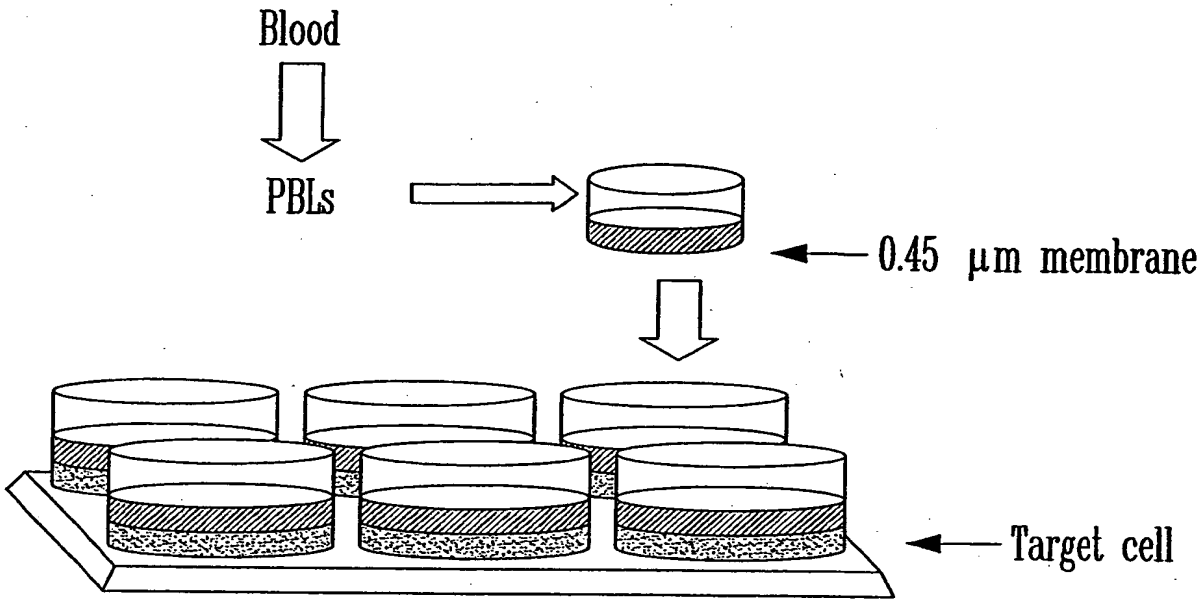


FIG_27A

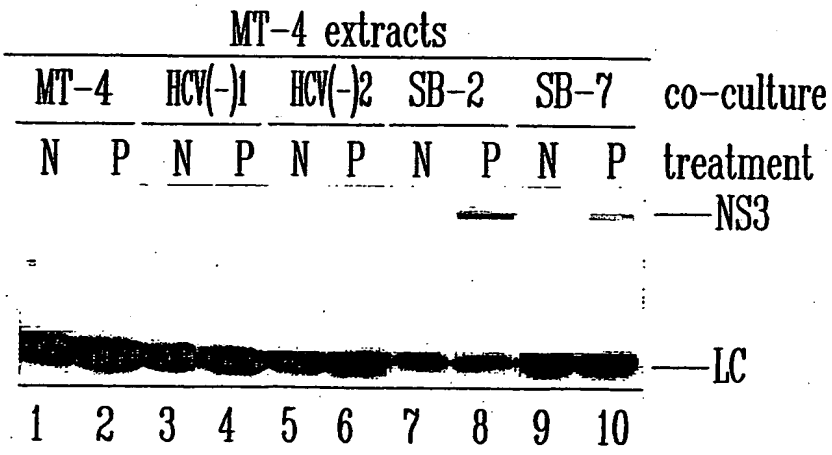


FIG_27B

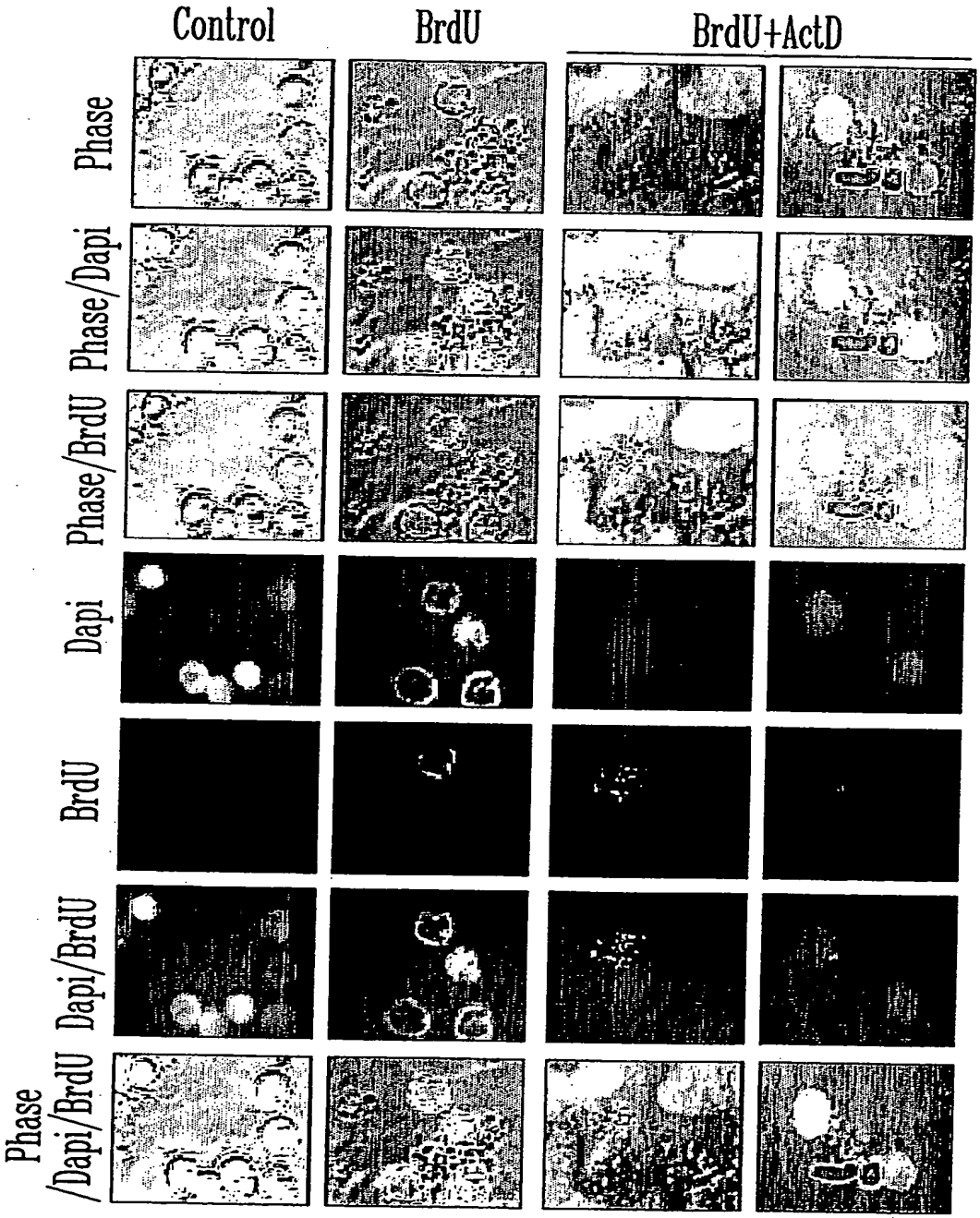

Control
BrdU
BrdU+ActD
Phase
Phase/Dapi
Phase/BrdU
Dapi
BrdU
Dapi/BrdU
Phase /Dapi/BrdU

Fig. 2B

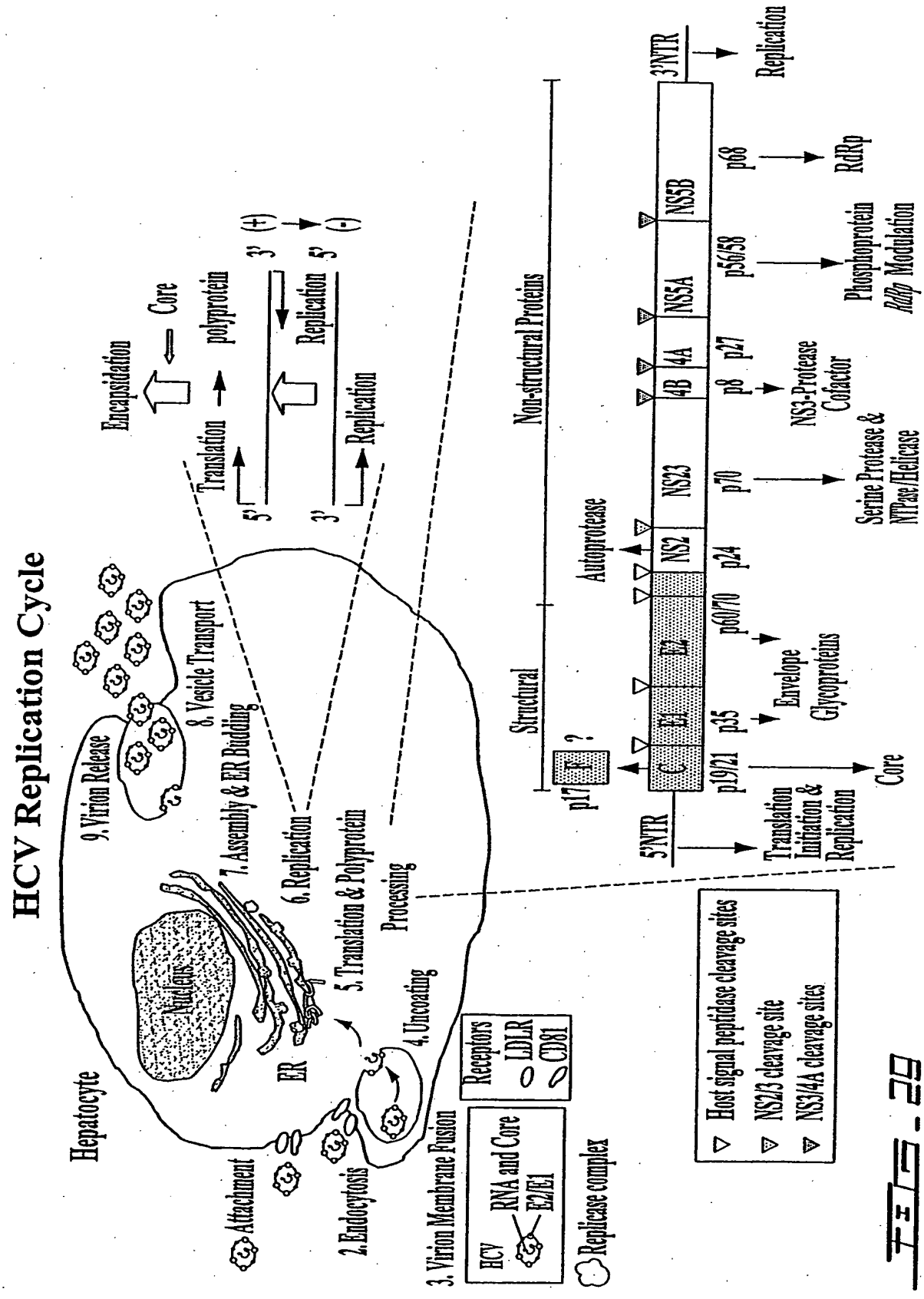

HCV Replication Cycle
Hepatocyte
1. Attachment
2. Endocytosis
3. Virion Membrane Fusion
4. Uncoating
5. Translation & Polyprotein Processing
6. Replication
7. Assembly & ER Budding
8. Vesicle Transport
9. Virion Release
Nucleus
ER
Receptors
LDLR
CD81
HCV
RNA and Core
E2/E1
Replicase complex
Encapsidation
Core
Translation
polyprotein
Replication
Non-structural Proteins
Structural
Autoprotease
5'NTR
C
E1
E2
NS2
NS23
4B
4A
NS5A
NS5B
3'NTR
p17
p19/21
p35
p60/70
p24
p70
p8
p27
p56/58
p68
Translation Initiation & Replication
Core
Envelope Glycoproteins
NS3-Protease Cofactor
Serine Protease & NTPase/Helicase
Phosphoprotein
RdRp
Replication
Host signal peptidase cleavage sites
NS2/3 cleavage site
NS3/4A cleavage sites

FIG - 29

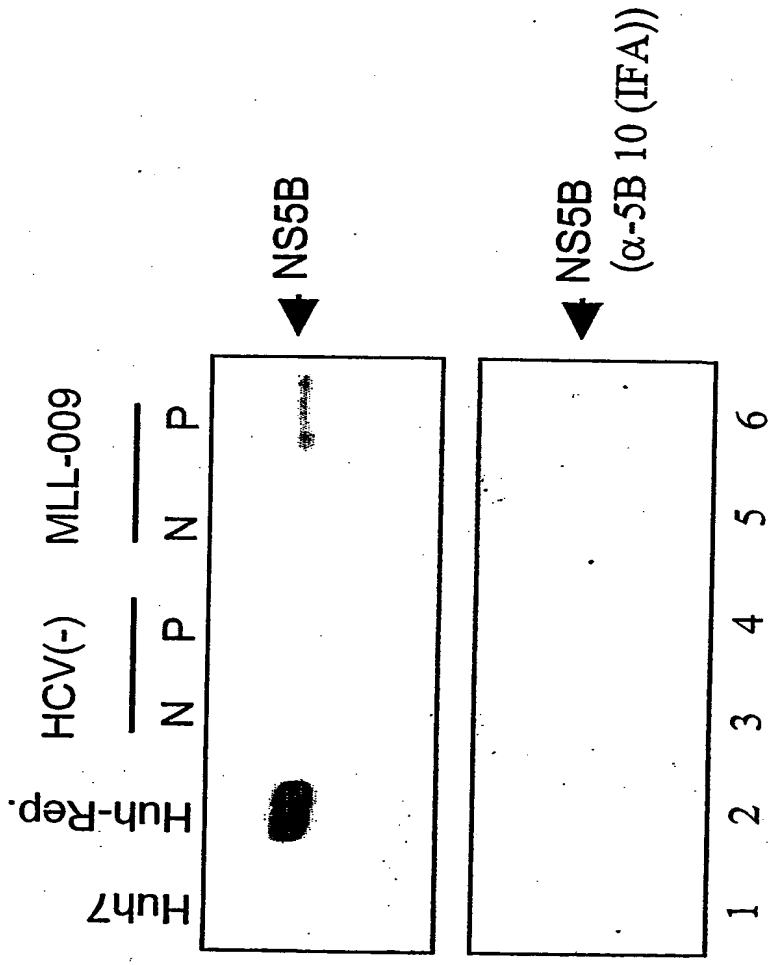


Fig. 30

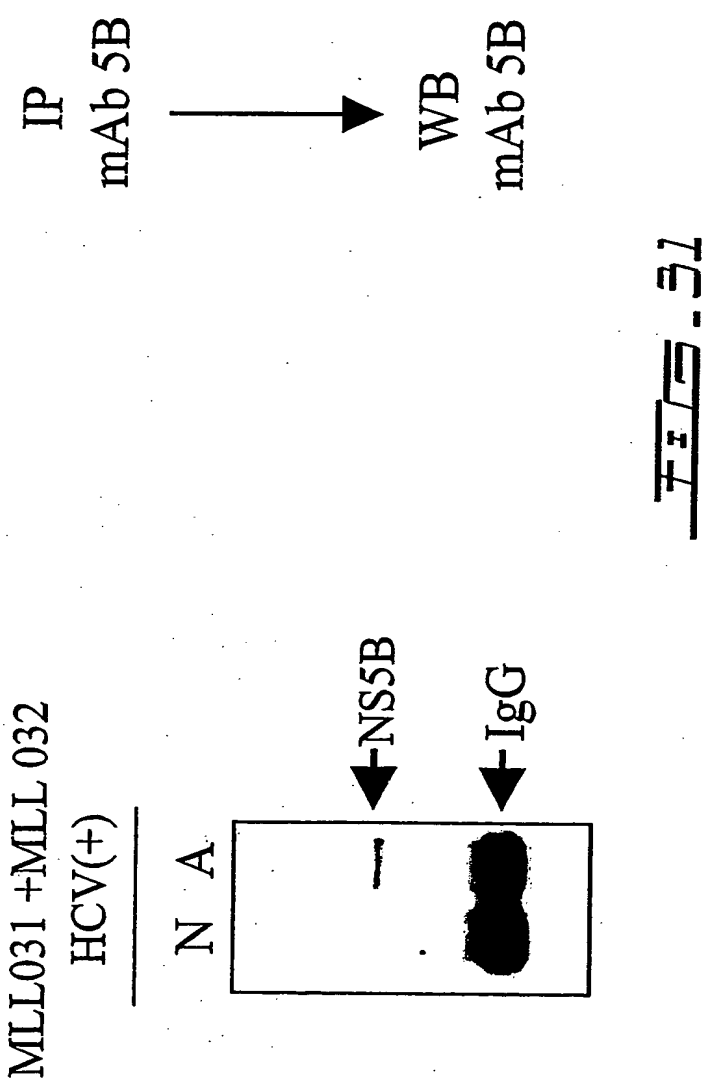


Fig. 31

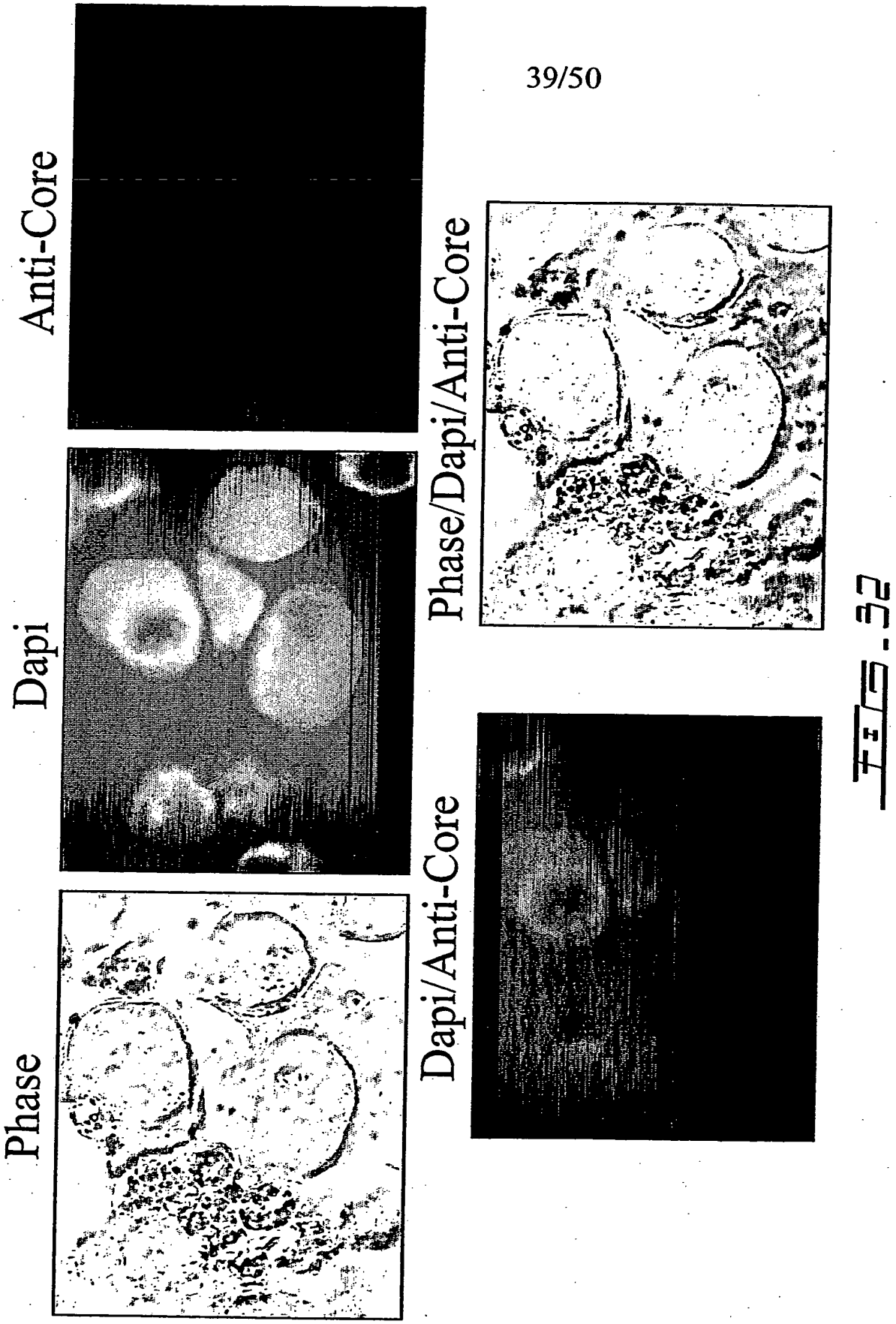


Fig. 32

FIG. 33

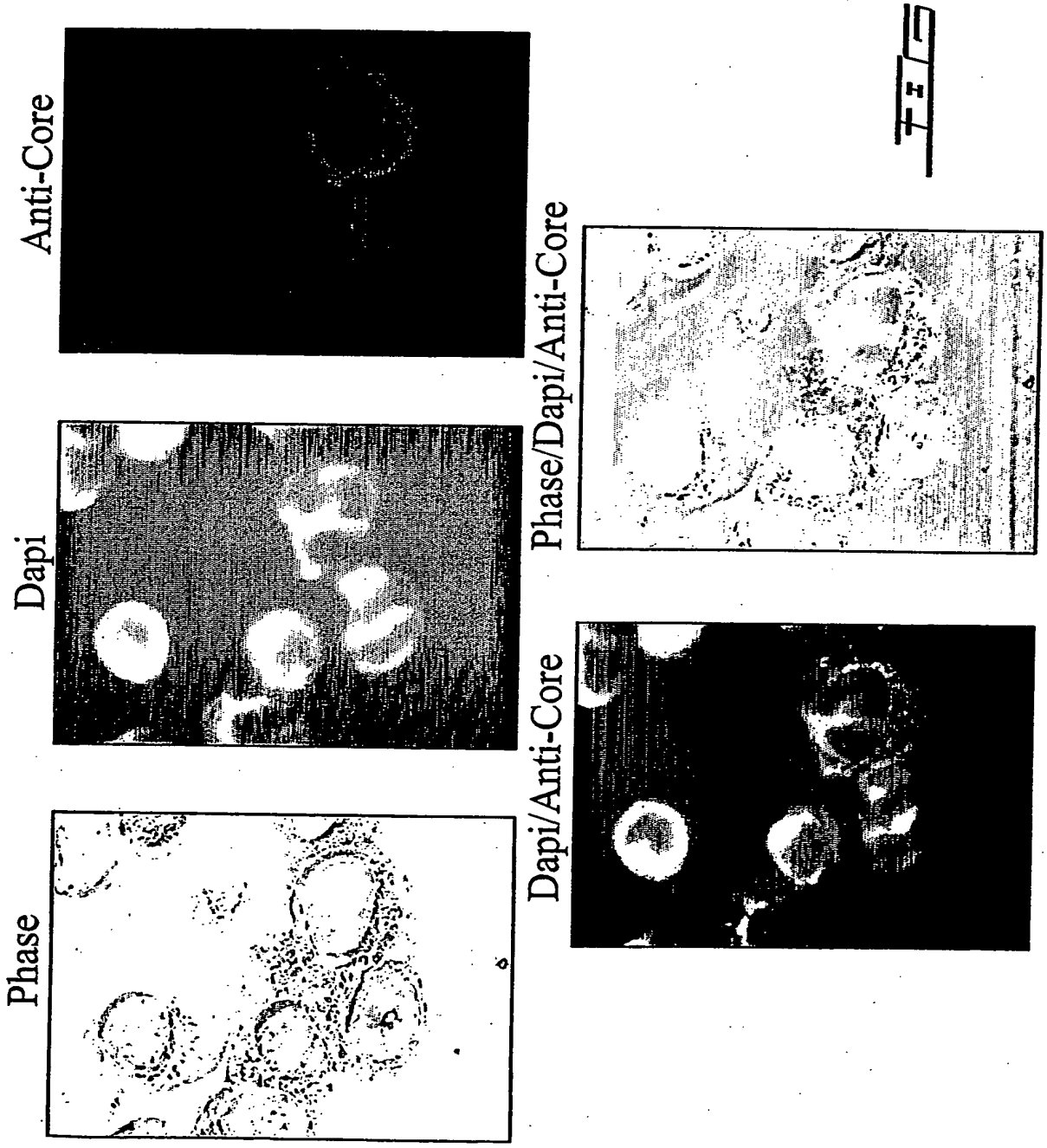
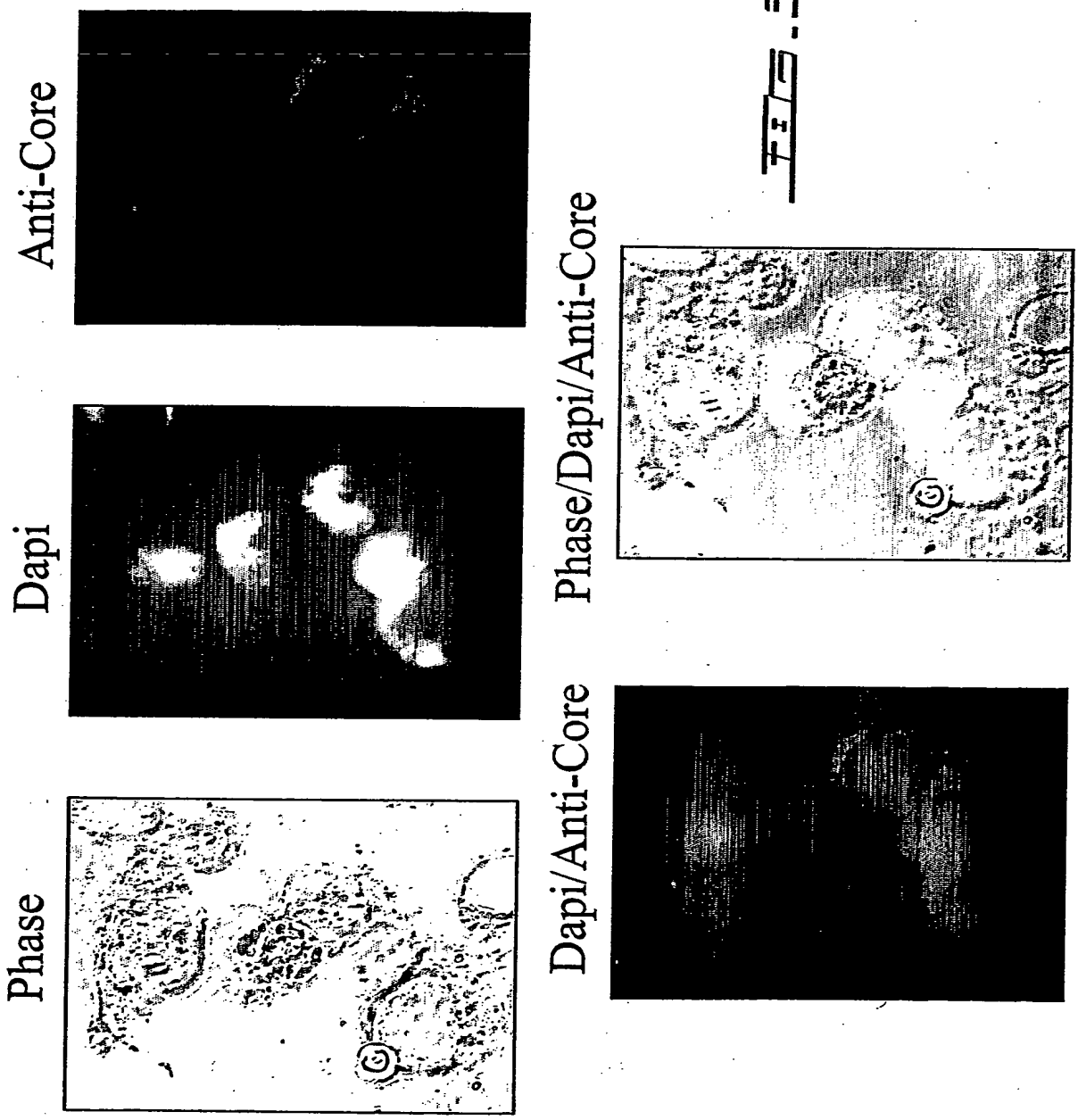


Fig. 34



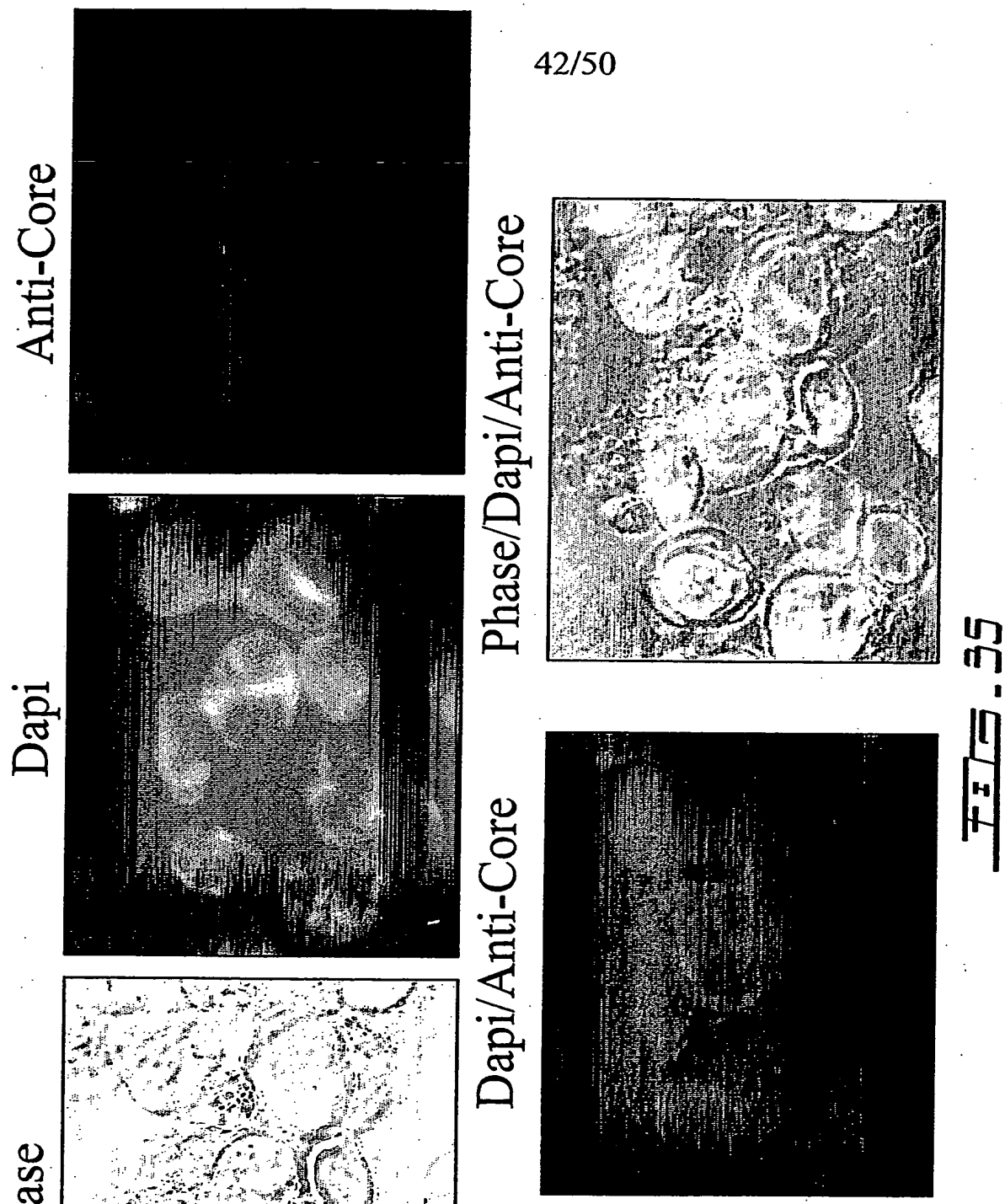


Fig. 35

Fig. 36C

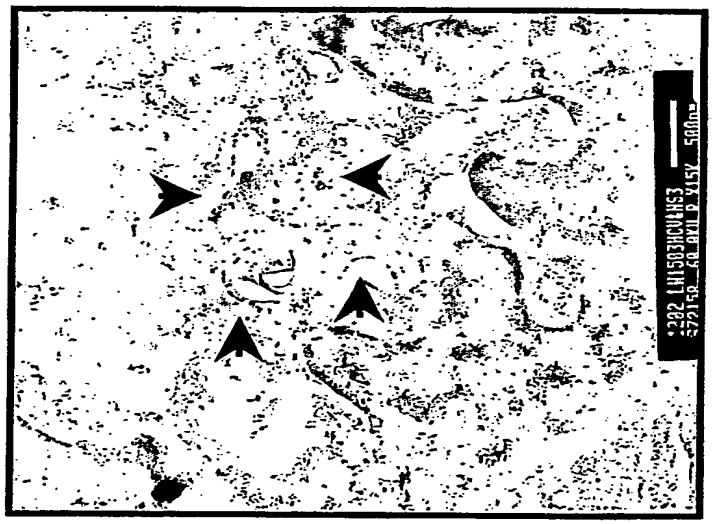


Fig. 36B

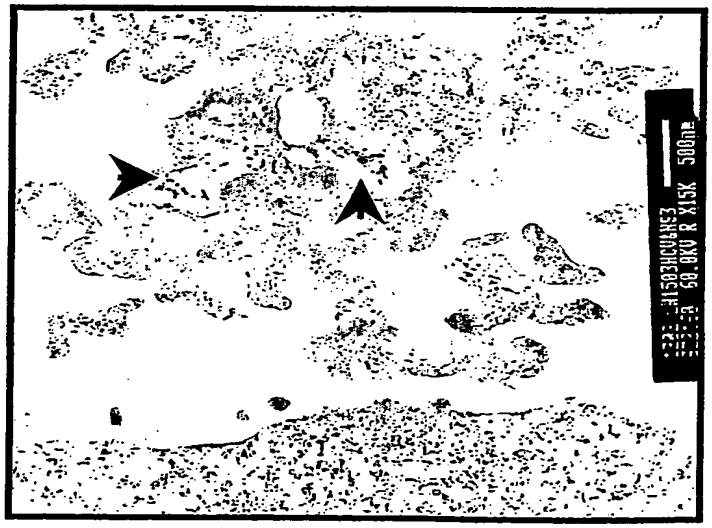


Fig. 36A

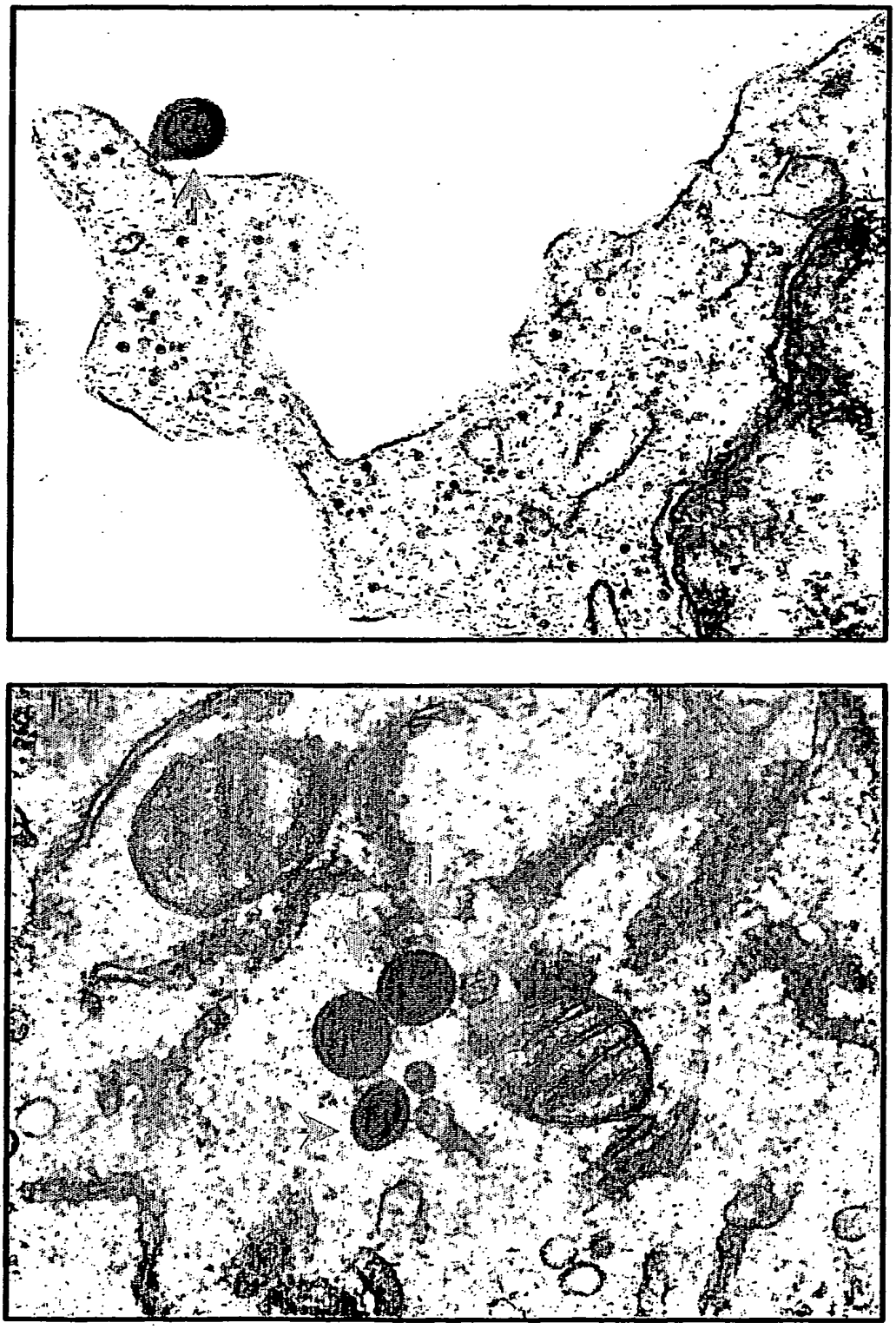


FIG. 37

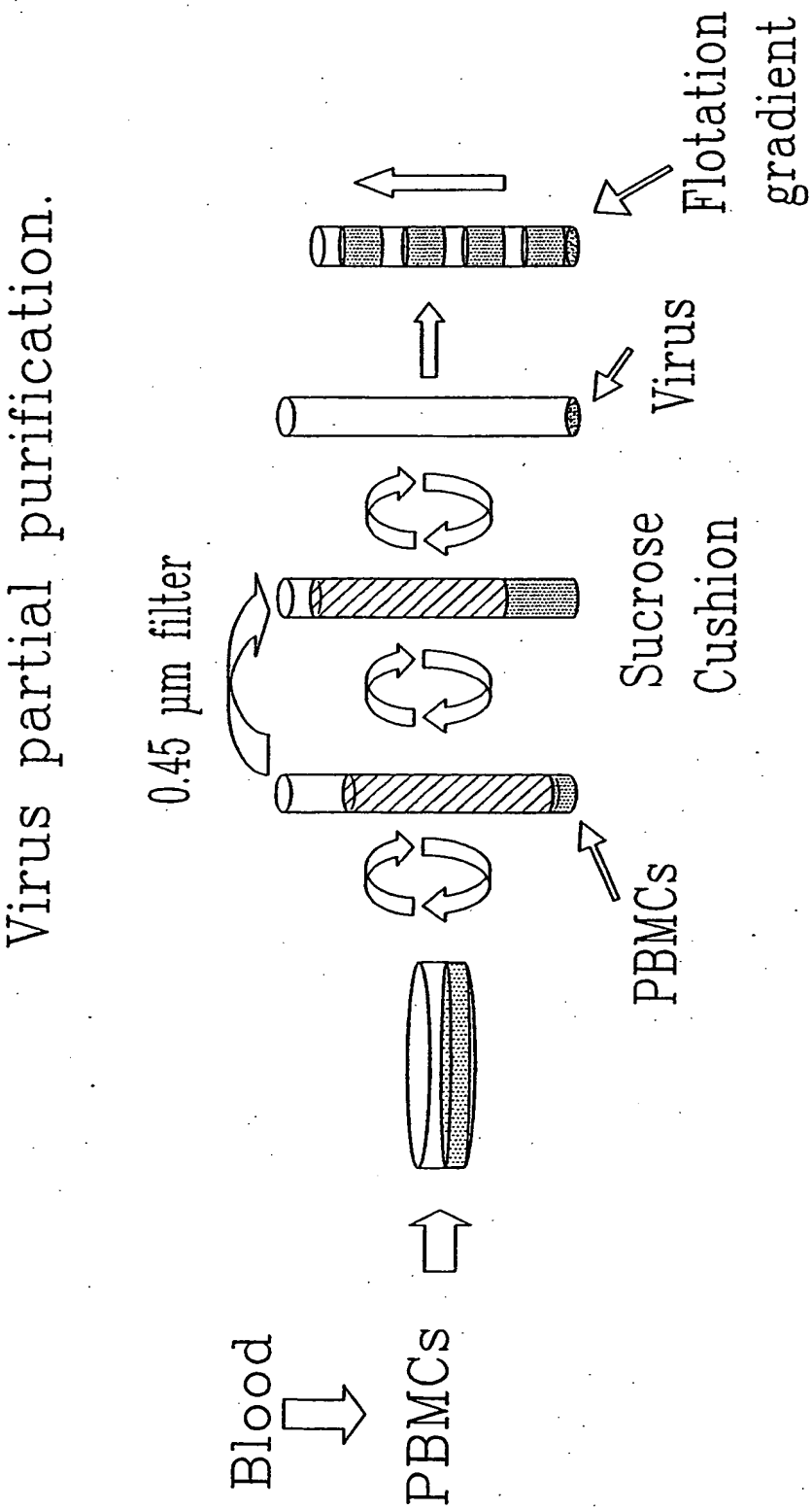

Virus partial purification.
Blood
PBMCs
0.45 µm filter
PBMCs
Sucrose Cushion
Virus
Flotation gradient

FIG. 3B

Density Range (g/ml)	Source	Reference
1.15-1.20	HCV-LP in VSV vector	J.Virol (2002) 76, 12325.
1.14-1.18	HCV-LP in insect cells	J. Virol (1998) 72, 3827.
1.12-1.17	Plasma chimps	J. Gen.Virol (1994) 75, 1755
1.09-1.21	Plasma chimps	J.Med.Virol (1991), 34, 206.
1.13-1.17	Plasma chimps	J.Virol (1993) 67, 1953
1.063-1.21	Serum infected donors	J Med Virol (2002) 68, 335

1.11-1.215	HCV(+) PBMCs	----------

Fig. 39

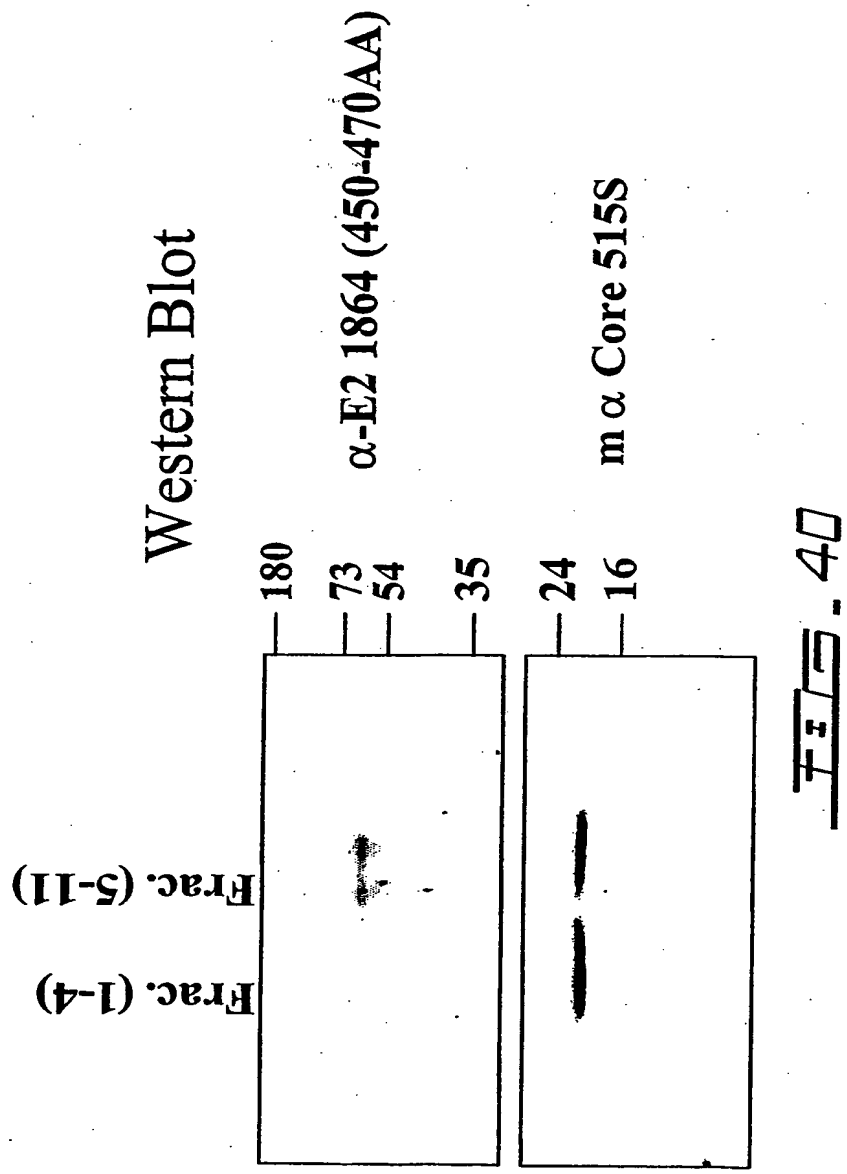

Western Blot
α-E2 1864 (450-470AA)
m α Core 515S
180
73
54
35
24
16
Frac. (5-11)
Frac. (1-4)

FIG. 40

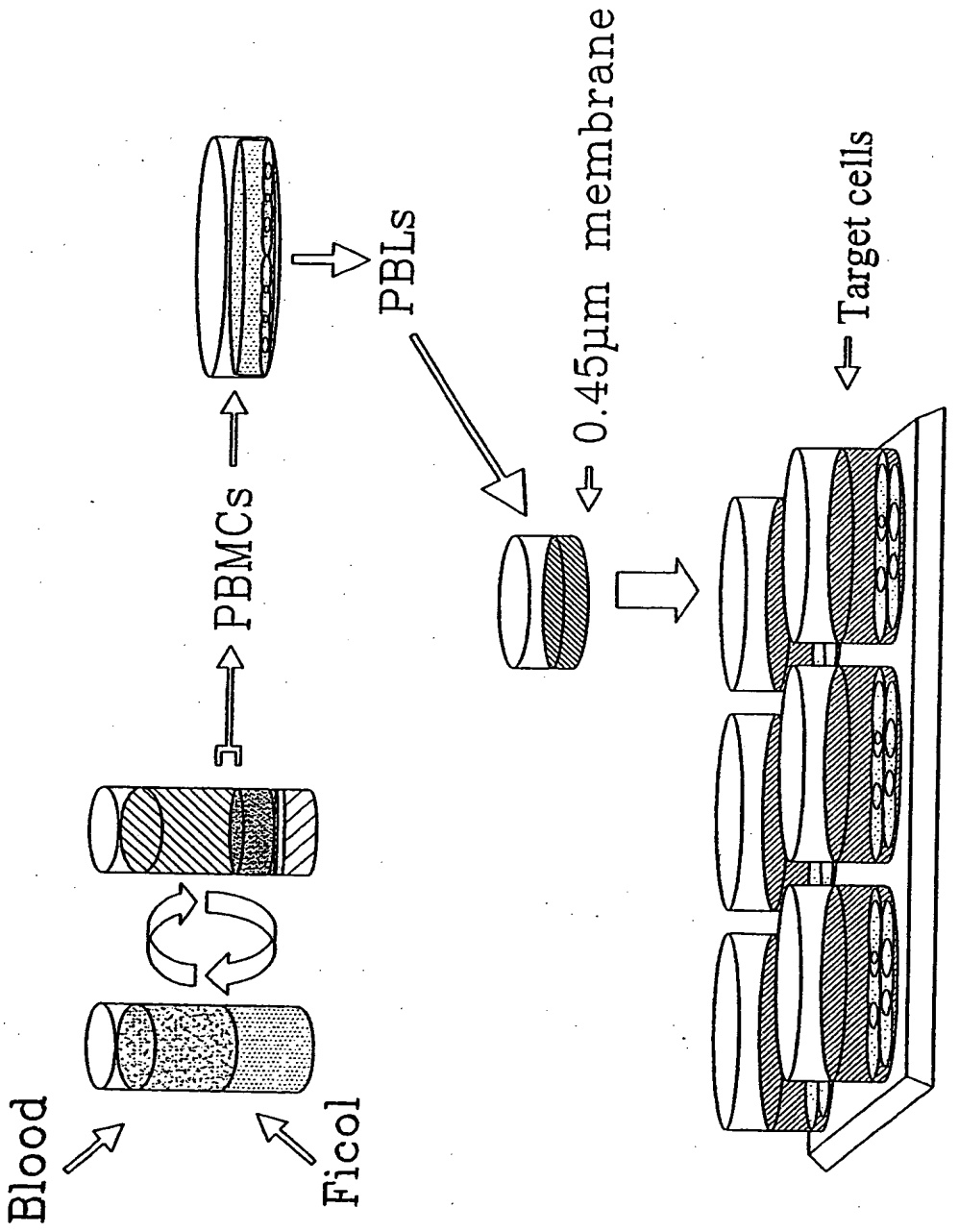


Fig. 41

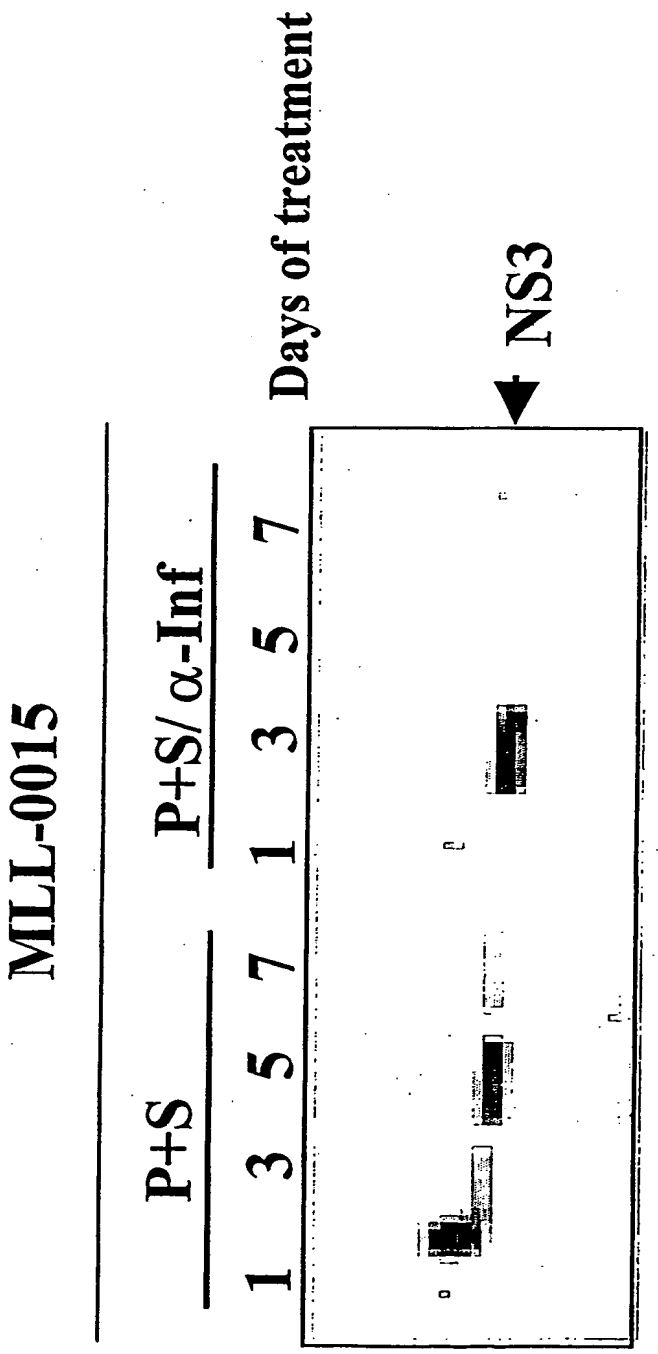


Fig. 42

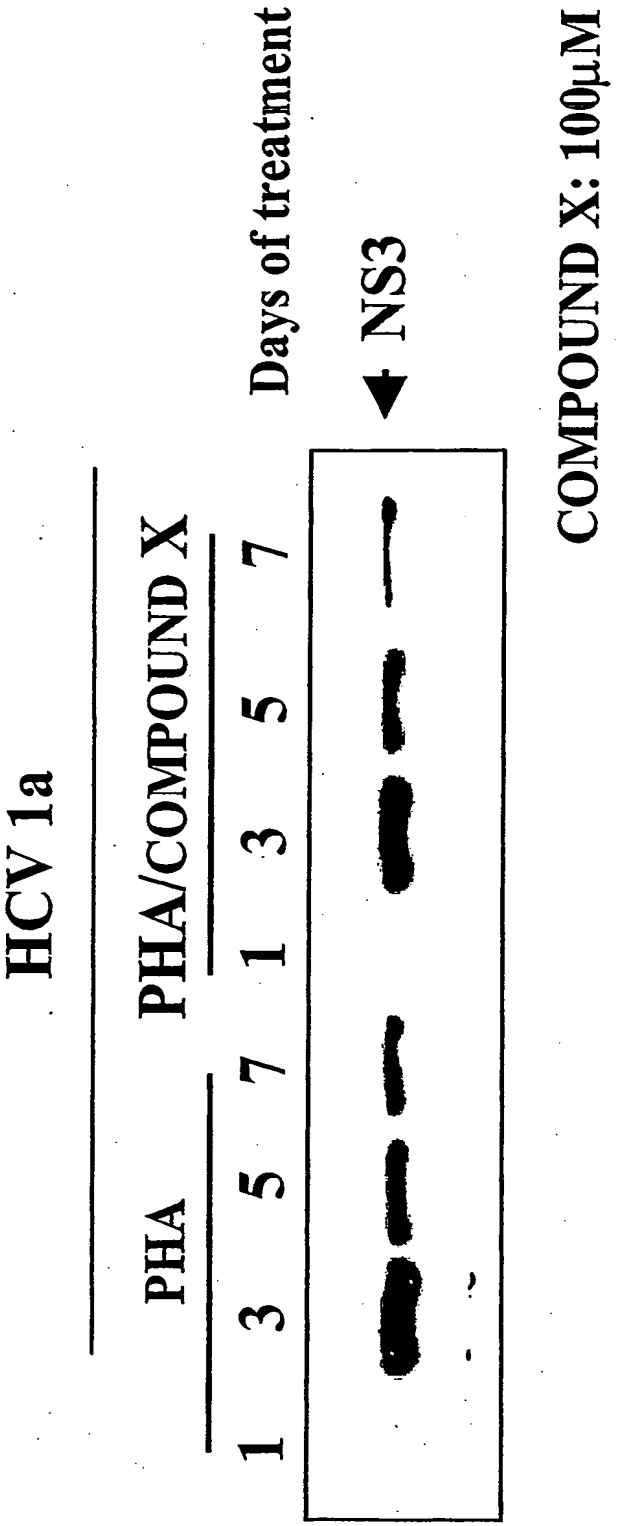

HCV 1a
PHA
PHA/COMPOUND X
1 3 5 7 1 3 5 7
Days of treatment
NS3
COMPOUND X: 100µM
m α-NS3 1G3D2

Fig. 43

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